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The synthesis of (\pm)-anatoxin-a

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The Synthesis of (\pm)-Anatoxin-a

Submitted by
Peter G. Vernon
for the degree of
Doctor of Philosophy
of the
University of Bath
1988

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To Julie

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SUMMARY

The silver(I)-initiated intramolecular cyclisation of δ -allenic amine derivatives to give 2,5-disubstituted pyrrolidines has been studied. It has been tentatively proposed that the stereoselectivity observed in this reaction, for certain amine derivatives, is due to unfavourable steric interactions in proposed intermediates.

This stereoselective route to cis-2,5-disubstituted pyrrolidines was then utilised as an important step in the synthesis of the neurotoxic alkaloid anatoxin-a. Two routes to the toxin were developed, both of which proceeded via the p-toluenesulphonyl derivative of 9-azabicyclo[4.2.1]nonan-2-one.

In an attempt to produce the toxin enantiospecifically the required pyrrolidine substrate was synthesised in an optically pure form. This was achieved by the silver(I)-initiated cyclisation of a suitably substituted allenic amine which had been enzymatically resolved. Unfortunately there was insufficient time to produce a sample of (+)-anatoxin-a from this material. However, work is currently underway within the group to achieve this.

ABBREVIATIONS

aq	aqueous
Ar	aryl
Boc	butoxycarbonyl
b.p.	boiling point
Bu	butyl
c	concentration
CI	chemical ionization
conc	concentrated
d	doublet
DBN	1,5-diazabicyclo[4.3.0]non-5-ene
DCB	2,6-dichlorobenzyl
d.e.	diastereomeric excess
DEAD	diethylazodicarboxylate
DME	1,2-dimethoxyethane
DMF	N,N-dimethylformamide
DMSO	dimethyl sulphoxide
E	trans
e.e.	enantiomeric excess
EI	electron impact
EtOAc	ethyl acetate
Eu(hfc) ₃	europium(III) tris(3-(heptafluoropropyl- hydroxymethylene)-d-camphorato)
¹ H nmr	proton nuclear magnetic resonance
HPMA	hexamethylphosphoramide
i-	iso

i.p.	intraparietal
l	path length
LD _{min}	minimum lethal dose
Lit	literature value
M	molar
m	multiplet
mAChR	muscarinic acetylcholine receptor
MCPBA	meta-chloroperoxybenzoic acid
m.p.	melting point
nAChR	nicotinic acetylcholine receptor
n-	normal
nOe	nuclear Overhauser effect
p	para
Pr	propyl
PTSA	para-toluenesulphonic acid
q	quartet
Ra/Ni	Raney nickel
RMBA	R-methylbenzylamine
s	singlet
t	triplet
t-	tertiary
THF	tetrahydrofuran
tlc	thin layer chromatography
TMS	trimethylsilyl
Tos	tosyl
VFDF	anabaena very fast death factor
Z	cis

CONTENTS

i	Copyright
ii	Dedication
iii	Acknowledgements
iv	Summary
v	Abbreviations
vii	Contents
1	Introduction
30	Results and Discussion
60	Conclusion
62	Recommendations for Further Work
63	Experimental
65	E-Pent-2-en-4-yn-1-ol (132)
66	Penta-3,4-dien-1-ol (133)
67	1-Bromopenta-3,4-diene (134)
67	Ethyl Penta-3,4-dienoate (136)
68	Penta-3,4-dien-1-ol (133)
69	1-Iodopenta-3,4-diene (137)
70	(1-Methoxyethyl)diphenylphosphine Oxide (177)
71	N-(phenylmethylene)glycine Ethyl Ester (138)
71	N-((4-methylphenyl)sulphonyl)-1-(penta-3,4-dienyl)-glycine Ethyl Ester (141)
74	N-((4-methylphenyl)sulphonyl)-1-(penta-3,4-dienyl)glycine (141 a)
75	Cis-5-ethenyl-1-((4-methylphenyl)sulphonyl)-proline Ethyl Ester (145)

- 76 Cis-5-ethenyl-1-((4-methylphenyl)sulphonyl)-
proline (148)
- 76 Cis-5-ethenyl-1-((4-methylphenyl)sulphonyl)-
proline Ethyl Ester (145)
- 77 Cis-5-(1-dueteroethylenyl)-1-((4-methyl-
phenyl)sulphonyl)proline Ethyl Ester (166)
- 78 N-((1,1-dimethylethoxy)carbonyl)-1-(penta-
3,4-dienyl)glycine Ethyl Ester (142)
- 80 1-((1,1-Dimethylethoxy)carbonyl)-5-ethenyl-
proline Ethyl Ester (146)
- 81 Cis-5-(2-hydroxyethyl)-1-((4-methylphenyl)-
sulphonyl)proline Ethyl Ester (168)
- 82 Cis-5-(2-bromoethyl)-1-((4-methylphenyl)-
sulphonyl)proline Ethyl Ester (173)
- 83 1-(Cis-5-(2-hydroxyethyl)-1-((4-methyl-
phenylsulphonyl)pyrrolidin-2-yl)-2-
(methysulphonyl)ethanone (169)
- 85 1-(Cis-5-(2-bromoethyl)-1-((4-methylphenyl)-
sulphonyl)pyrrolidin-2-yl)-2-(methyl-
sulphonyl)ethanone (170)
- 86 9-((4-Methylphenyl)sulphonyl)-3-(methyl-
sulphonyl)-9-azabicyclo-[4.2.1]nonan-2-
one (171)
- 87 9-((4-Methylphenyl)sulphonyl)-9-azabicyclo-
[4.2.1]nonan-2-one (174)
- 88 (E+Z)-2-(Methoxyethylidenyl)-9-((4-methyl-
phenyl)sulphonyl)-9-azabicyclo[4.2.1]non-
ane (179)

- 90 ($\alpha+\beta$)-2-Acetyl-9-((4-methylphenyl)sulphonyl)-9-azabicyclo-[4.2.1]nonane (188)
- 91 (E+Z)-2-(1-Methoxyethylidene)-9-azabicyclo-[4.2.1]nonane-9-carboxylic Acid 1,1-Dimethylethyl Ester (186)
- 92 ($\alpha+\beta$)-2-Acetyl-9-azabicyclo[4.2.1]nonane-9-carboxylic Acid 1,1-Dimethylethyl Ester (189)
- 93 (E+Z)-2-(Methoxymethylene)-9-((4-methylphenyl)sulphonyl)-9-azabicyclo[4.2.1]nonane (181)
- 95 (E+Z)-2-(Methoxymethylene)-9-azabicyclo-[4.2.1]nonane (182)
- 96 (E+Z)-2-(Methoxymethylene)-9-azabicyclo[4.2.1]-nonane (182)
- 97 (E+Z)-2-(Methoxymethylene)-9-azabicyclo[4.2.1]-nonane-9-carboxylic Acid 1,1-Dimethylethyl Ester (183)
- 98 2-Carboxaldehyde-9-((4-methylphenyl)sulphonyl)-9-azabicyclo[4.2.1]non-2-ene (190)
- 99 2-Carboxaldehyde-9-azabicyclo[4.2.1]non-2-ene-9-carboxylic Acid 1,1-Dimethylethyl Ester (191)
- 100 2-Acetyl-9-azabicyclo[4.2.1]non-2-ene-9-carboxylic Acid 1,1-Dimethylethyl Ester (192)
- 101 2-Acetyl-9-azabicyclo[4.2.1]non-2-ene Hydrochloride (15)

102	(2R,5S)-2,5-Dihydro-3,6-dimethoxy-2-(1-methylethyl)-5-(penta-3,4-dienyl)pyrazine (201)
103	(2R,5S)-2,5-dihydro-3,6-dimethoxy-2-(1-methylethyl)-5-(penta-3,4-dienyl)pyrazine (201)
104	(1R)-N-((4-Methylphenyl)sulphonyl)-1-(penta-3,4-dienyl)glycine Ethyl Ester (203)
106	(1R)-N-((4-Methylphenyl)sulphonyl)-1-(penta-3,4-dienyl)glycine(204)
106	Cis-(2R,5S)-1-((4-methylphenyl)sulphonyl)-5-ethenylproline (206)
108	Appendix 1
111	Appendix 2
114	Publications
115	References

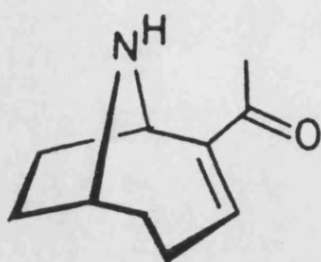
INTRODUCTION

Species of marine algae have been gathered and used for food in Japan and China for centuries. This has prompted a great deal of research in recent times into developing methods for cultivating, harvesting and drying both seaweeds and freshwater micro-algae for use as both human and livestock food sources. A class of blue-green freshwater micro-algae, known as the cyanophytes, are a potentially excellent source of food. Cyanophytes have a high protein content, a good digestibility and can be cultured quite rapidly on by-products or waste sources of nutrients. A serious problem in this area of research is that toxic strains of several species of bloom forming cyanophytes are known to occur which are morphologically indistinguishable from non-toxic strains.

Poisonings of livestock and other animals due to ingestion of toxic cyanophytes are regularly reported in western Canada, mid-western United States and similar climatic regions throughout the world. The three most common bloom forming species which are known to produce toxic strains are *Microcystis aeruginosa* Kütz. emend Elenkin, *Anabaena flos-aquae* (Lyngb.) de Bréb. and *Aphanizomenon flos-aquae* (L.) Ralfs. These have been shown to be responsible for most of the observed poisonings^{1,2,3}.

Rose was among the first to investigate toxic blooms of *Anabaena flos-aquae*⁴. He described how, in the autumn of 1952, an extremely heavy growth of a poisonous strain of the alga developed at Storm Lake in Iowa. Due to prolonged "Indian Summer" conditions the growth did not disappear prior to the autumn migration of waterfowl. As a result a large number of animals were killed including over six thousand migrating birds. Tests undertaken by Rose ruled out botulism as a possible cause of death but showed it to be a result of ingestion of the healthy living alga.

The first isolation of both toxic and non-toxic clones of *Anabaena flos aquae*, cultured from a toxic bloom of the species, was reported by Gorham and co-workers⁵. The same group later described conditions for the large scale culture of a bacteria free clone from these isolates⁶. They called the exotoxin obtained from these cultures *Anabaena* Very Fast Death Factor (VFDF).



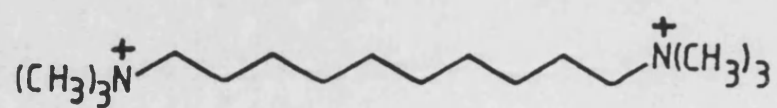
Anatoxin-a

(1)

The crystal structure of the N-acetyl derivative of VFDF was determined by Huber in 1972⁷. Later Edwards published a spectroscopic study of the toxin as well as an efficient method for extracting VFDF from the parent alga⁸. It was around this time that the toxin became known as anatoxin-a, its systematic name being 2-acetyl-9-azabicyclo[4.2.1]non-2-ene (1). Anatoxin-a has a molecular weight of 166 with an LD_{min} (i.p. mouse) of 0.25mg kg⁻¹; four to five minutes survival time⁸.

Laboratory experiments with selected animals showed the toxin to be rapidly absorbed by the oral route. Death was seen to be primarily caused by respiratory arrest since with artificial respiration normal body functions, such as blood pressure, could be maintained for long periods of time. However, artificial respiration was not a good method of detoxification due to the long duration of action of the toxin⁹.

Preliminary pharmacological investigations showed that the main effect of the toxin was production of a sustained postsynaptic depolarising neuromuscular blockade^{9,10,11}. The short survival times were indicative of a neuromuscular poison, but the key to the depolarising properties of the toxin came from the observed opisthotonus and muscle rigidity in avian species. These signs were indistinguishable from those obtained

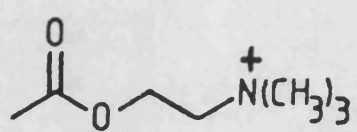


Decamethonium

(2)

by earlier workers for the depolarising compound decamethonium (2)¹².

The propensity of anatoxin-a to induce opisthotonus and muscle rigidity was used in conjunction with differences in survival time, salivation, lachrymation and chromodacyrrhoea to identify three and possibly five additional *Anabaena* toxins¹³. These were named anatoxin-b, -c and -d and were produced by clones of *Anabaena flos-aquae*. Clones which produced salivation as well were designated anatoxin-a(s) and -b(s). It is possible that there may be structural similarities between these and known toxins or that some or all may be mixtures. Short survival times suggest that types -b and -d are likely to contain low molecular weight alkaloids. Anatoxin-c was found to be a mixture of two low molecular weight toxins. One is a peptide with hepatotoxic properties and the other caused death in mice by respiratory arrest which suggests it may be a neurotoxin.



Acetylcholine

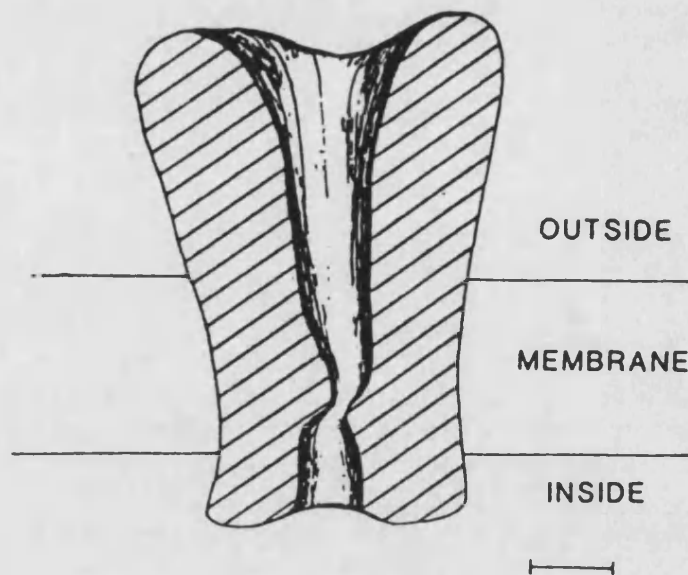
(3)

The nicotinic acetylcholine receptor (nAChR)¹⁴ is found at the neuromuscular endplate, in the central nervous system and in a variety of peripheral ganglia and tissues. The function of the receptor is to recognise and bind specifically the neurotransmitter acetylcholine (3) released from a nerve ending. Located in the postsynaptic membrane, the receptor has to convert this chemical signal into an electrical effect. This consists of a depolarisation of the postsynaptic membrane due to an influx of cations through an ion channel.

The nAChR has been shown to be a pentameric protein complex composed of five polypeptide chains of four types. The resulting structure $\alpha_2\beta\gamma\delta$ ¹⁵ was shown to have these subunits arranged in the order $\beta\alpha\delta\gamma\alpha$ ¹⁶ and forms a ring like structure that spans the membrane (scheme 1).

There is little doubt that the ion channel is truly an integral part of the $\alpha_2\beta\gamma\delta$ pentamer. Channel opening is brought about by the binding of two acetylcholine molecules to the nAChR, probably one to each of the two α subunits. This binding results in a change of conformation of the pentameric protein causing the ion channel to widen and allow cations to pass through it¹⁴. The channel is a water-filled pore and permeating ions probably interact with water molecules rather than the

6



Scale cut-away drawing depicting the approximate dimensions of the nACHR and its position in the membrane. The taper of the channel and its constriction are arbitrary. The scale bar represents 2.0 nm.

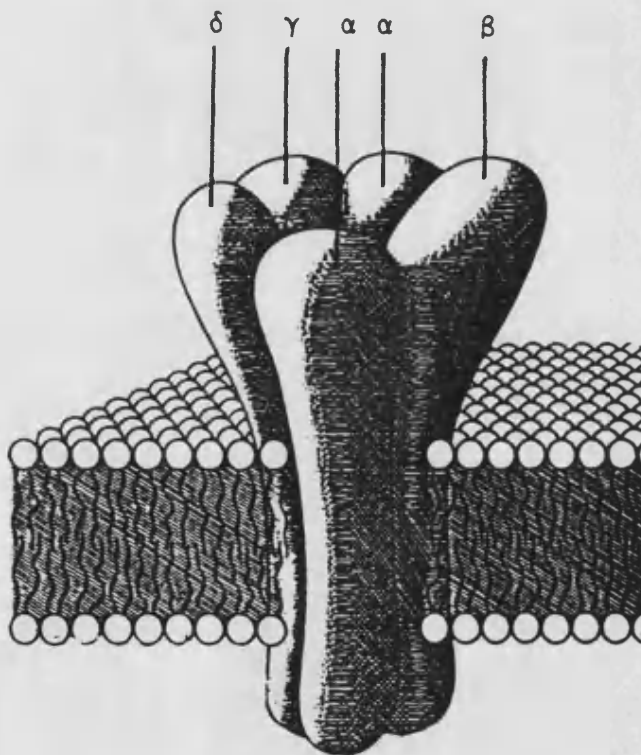


Diagram showing 5 polypeptide chains, which form the nACHR, spanning the membrane.

SCHEME 1

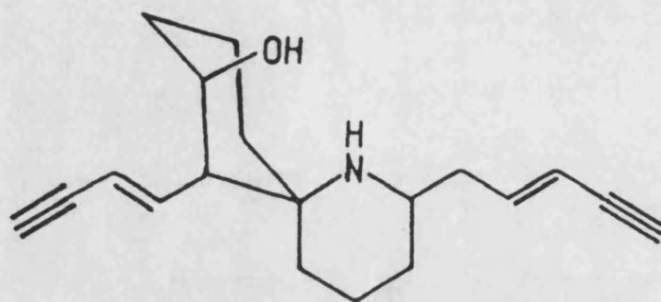
protein side chains.¹⁷

There are three main types of cholinergic effector molecules: agonists, competitive antagonists and non-competitive antagonists. Agonists, like the neurotransmitter acetylcholine, bind to the nAChR and activate it. Competitive antagonists combine too, but exert no effect other than displacing agonists from their binding sites. Non-competitive antagonists do not displace the agonist but still block activation of the nAChR probably by a more direct interaction with the ion channel. Prolonged interaction of an agonist with the nAChR, seconds or longer, leads to receptor desensitisation¹⁸ accompanied by channel closing. That is, a progressive decline in the number of open channels combined with up to a two fold increase in affinity of the receptor for the agonist.¹⁹ This phenomenon is observed more in experimentally induced rather than natural release of acetylcholine. Analysis of the concentration dependence of desensitisation and of recovery indicates that it occurs because the receptor conformation binding an agonist with highest affinity is not the open channel conformation but an inactivated desensitised one. It is thought that the agonist remains bound to the nAChR once it is activated or desensitised and the mean open time of the channel depends on temperature, membrane potential and the agonist.¹⁴ Thus receptor action can be described as

transitions between the following states; firstly a resting state (channel closed, affinity for agonist low), secondly an activated state (channel open, affinity for agonist unknown) and finally a desensitised state (channel closed, affinity for agonist high). The different states are thought to correspond to different conformations of the pentameric protein.

Nicotinic agonists such as anatoxin-a are generally small organic cations (often quaternary amines) that react with the recognition site of the nAChR and permit the associated ion channel to open. As ions cross the muscle membrane the end plate region of the fibre depolarises and, in multiply innervated fibres, the fibre undergoes contracture. Potencies of agonists are frequently compared by the force of contracture a given concentration will induce in frog rectus abdominous muscle or by the depolarisation (measured by intracellular microelectrodes) induced in individual muscle fibres. These potencies are composite functions of more elementary parameters such as the opening frequency, the lifetime and the conductance of the nAChR ion channel.¹⁴

The distinction between competitive and non-competitive antagonists is seen most clearly in biophysical studies. Effects associated with ion channel blockade include a non-linearity of the end



Histrionicotoxin

(4)

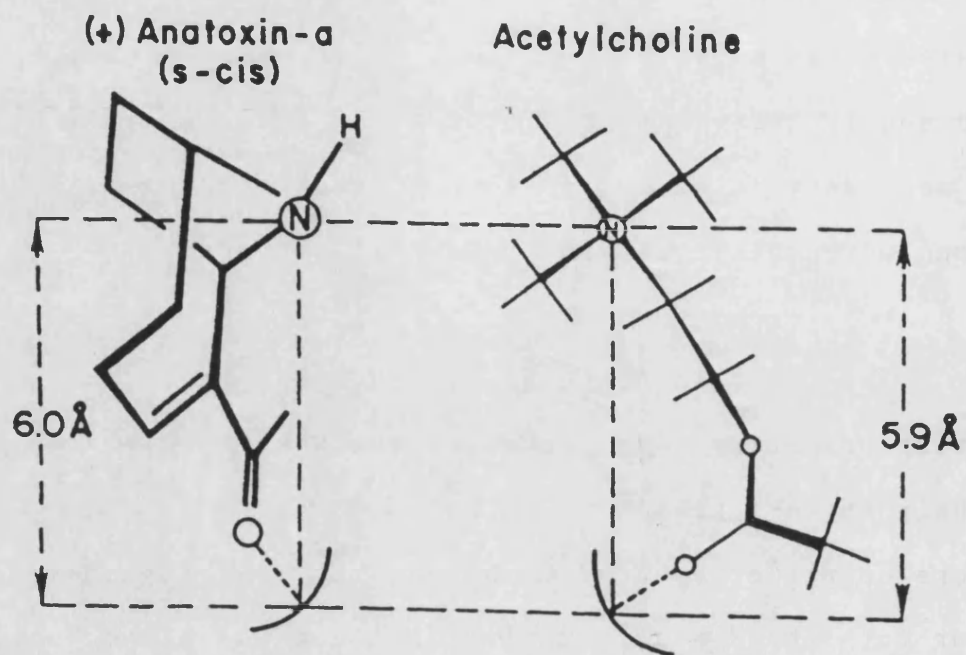
plate current to membrane potential relationship and alteration in the lifetime of the individual ion channels. Non-competitive antagonists that interact with the channel and not the receptor have been identified, an example being histrionicotoxin (4). Although these compounds have been termed channel probes, the precise position of their binding has not been established. They may bind to a site adjacent to the channel rather than directly to the channel itself. There is even evidence to suggest that different probes bind to different sites on the nAChR complex.

Cholinergic ligands such as anatoxin-a have been shown to affect the equilibrium binding of histrionicotoxin and vice versa²⁰. Anatoxin-a stimulates the binding of ion channel blockers possibly because the channel blocker binds only to sites associated with the channel that are exposed upon activation. Tests on this property²¹ along with electrophysiological studies²² showed anatoxin-a to be a potent depolarising and blocking agent acting at the neuromuscular junction. In addition to this the toxin appeared to have some presynaptic action which resulted in the reduction of the release of acetylcholine¹⁰.

It has been proposed that the nAChR donates a hydrogen bond to the agonist (the hydrogen bond acceptor in anatoxin-a is the carbonyl group). An

anionic centre of the nAChR also forms an electrostatic bond with the cationic centre of the agonist (the amine in anatoxin-a)²³. The pKa of anatoxin-a has been established as 9.36 in aqueous solution at physiological pH and is present as the protonated form in >99%.²⁴ As a secondary amine it can form stronger coulombic bonds than more bulky quaternary substituted agonists.

The bicyclic structure of anatoxin-a is thought to exist primarily with the seven membered ring in the twist chair conformation²⁴. The fate of the enone system is more complicated. Crystal structures have been determined for both the N-acetyl derivative⁷ and the hydrochloride salt²⁴ of anatoxin-a and in both cases the enone is in the s-trans conformation. This is probably a result of crystal packing forces as both force field calculations²⁴ and infra red spectroscopy⁸ predict the s-cis form to be favoured over the s-trans. ¹H nmr nOe studies however, show no real preference for either conformation²⁴. The s-cis form, being sterically less demanding, is thought to be more stable in solution. The distance between the nitrogen atom and the carbonyl oxygen of the agonist has been shown to be very important. The ideal distance, based on the conformational analysis of several agonists and antagonists, has been predicted to be 5.9Å by Beers and Reich²³. This distance in anatoxin-a, when the enone adopts the s-cis conformation, is 6.0Å giving

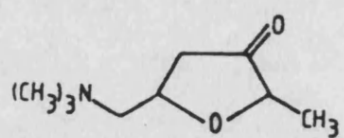


SCHEME 2

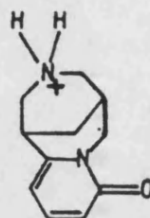
further evidence that the s-cis conformation activates the nAChR (scheme 2).

Stereospecificity is evidenced at the nAChR by the much greater potency of natural (+)-anatoxin-a to (-)-anatoxin-a (>150 fold)²⁵. The activity of synthetic unnatural (-)-anatoxin-a is so low that it may be due to traces of (+)-anatoxin-a in the sample tested. Stereospecificity is thought to arise as a result of the nAChR recognising a plane defined by the hydrogen bond acceptor of the agonist. In the case of anatoxin-a this would be the carbonyl group and the groups two substituents. The anionic centre of the nAChR is set off this plane and these conditions along with the importance of the distance between the two centres in the agonist is sufficient to establish chirality at the recognition site.

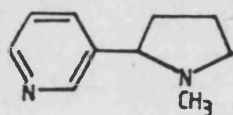
This also explains the effect of quaternisation on agonist potency. The two enantiomers of the nAChR agonist muscarone (5) are nearly equipotent. This is a result of the quaternary nitrogen atom of muscarone (5) lying on the carbonyl plane so that the N-methyl groups, which probably bear the positive charge, can project on either side of this plane regardless of the enantiomer. Therefore this hypothesis explains the function of quaternisation as a forced displacement of a cationic centre out of the carbonyl plane. In polycyclic nicotinic



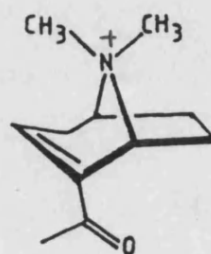
Muscarone
(5)



Cystine
(6)



Nicotine
(7)



Ferunginine
(8)

agonists such as anatoxin-a (1), quaternisation is unnecessary because the ring system separates the cationic region from the carbonyl plane. However, although anatoxin-a (1), cystine (6) and nicotine (7) support this theory, it is not clear why N-methylation so markedly enhances the activity of (-)-ferruginine (8)²⁵.

Concentration response studies showed (+)-anatoxin-a to be the most potent nAChR agonist of the several studied. (+)-Anatoxin-a elicits muscle contracture and is about eight times more potent than acetylcholine after acetylcholine-esterase inhibition²⁶. This is due to the toxin having a 3.6 fold greater affinity for the agonist binding site and hence causing a greater frequency of channel opening. Binding of two agonist molecules to α -chains of the receptor is required to activate a single channel. If there is no allosteric co-operativity, the fraction of doubly liganded sites is the square of the fraction of sites bound. Thus thirteen times more receptors would be doubly liganded and therefore activated with (+)-anatoxin-a than with acetylcholine. This is greater than the observed contracture ratio but does show that the higher binding affinity of (+)-anatoxin-a, which elicits channel opening and enhances histrionicotoxin binding, is a major contributing factor to the greater potency of the

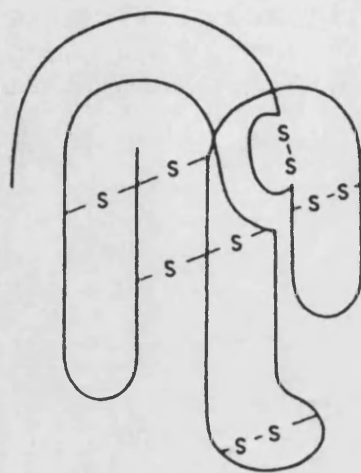
toxin. Another factor is that despite the greater binding affinity of (+)-anatoxin-a, the onset of desensitisation due to the toxin occurred at a two fold slower rate than that due to acetylcholine thus enhancing the relative potency of the toxin.

(+)-Anatoxin-a does have some destabilising features that restrict its potency. One difference between (+)-anatoxin-a and acetylcholine activated channels was an increased frequency of short gaps and a shortened open channel time. The shorter ionic burst time actually decreased the current flow, countering the effect of the toxins higher binding affinity. The short gaps may be a result of normal closures of ion channels followed by reopening without dissociation of the agonist having taken place. This process would account for the transition between open and closed channels being more rapid for (+)-anatoxin-a activated receptors than acetylcholine activated receptors, despite the fact that (+)-anatoxin-a has been shown to dissociate more slowly from the nAChR than acetylcholine²⁶. The greater rigidity of (+)-anatoxin-a may be largely responsible for the toxins high affinity for the nAChR but may also be responsible for the greater rate of ion channel closure. During ionic channel activation, conformational changes of the receptor macromolecule may be accommodated by the flexibility of many agonists. In contrast, the rigidity of (+)-anatoxin-a could destabilise the open conformation.

Another destabilising factor may be that intramolecular hydrogen bonding may encourage movement between the s-cis and the s-trans conformations of the enone system. Also in physiological solution it is probably protonated (+)-anatoxin-a that binds to the receptor and activates the ion channel, thus proton dissociation may enhance channel closure.

Biochemical results and biophysical measurements of synaptic transmission at the neuromuscular junction show that at physiologically active concentration (+)-anatoxin-a does not block the open ion channel or produce a voltage dependent blockade of the closed channel²². However, if (+)-anatoxin-a underwent some form of non stereospecific blockade of the nAChR, this would account for the potency ratio of (+)- to([±])-anatoxin-a slightly exceeding 2:1 (actually 2.5:1)²⁶.

The long duration of action of (+)-anatoxin-a is a result of three factors. Firstly (+)-anatoxin-a undergoes prolonged neuromuscular blockade, secondly the toxin reduces the release of acetylcholine (3) due to presynaptic action and finally (+)-anatoxin-a does not contain an ester linkage which may be subject to enzyme hydrolysis.



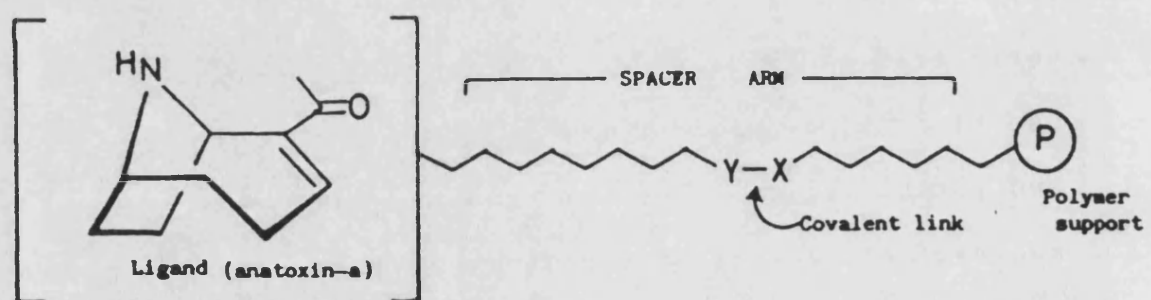
α -Bugarotoxin

(9)

Schematic diagram showing the shape of the peptide chain and the disulphide bridges.

Despite the wealth of knowledge about the nAChR in the peripheral nervous system, little is known about the nAChR in the central nervous system. The reason for this is that the latter are present only in low concentration and most attention has focused on the more abundant muscarinic acetylcholine receptor (mAChR) in the central nervous system. Also the great success in isolating and characterising the peripheral nAChR depended to a large extent on the use of [¹²⁵I] α -bungarotoxin (9) as a very specific and potent ligand. This probe has proved less useful and even misleading in the study of the neuronal nAChR.

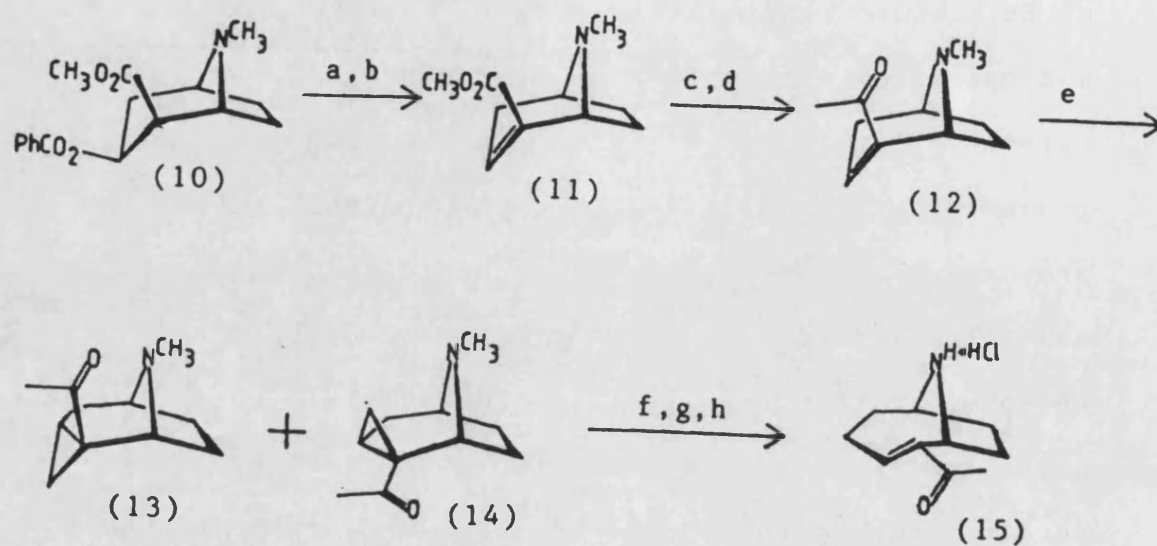
It is the neuronal nAChR which nicotine is thought to affect causing tobacco smoking to be habit forming²⁷. Also, post-mortem examination of brain tissue from sufferers of Alzheimer's disease suggest that the neuronal nAChR may play a role in the pathogenesis or clinical symptoms of the disease^{28,29}. Thus an understanding of the molecular constraints at the recognition site of the brain nAChR may facilitate selective therapeutic drug design to alleviate some aspects of this dementia.



SCHEME 3

(+)-Anatoxin-a has been shown to act weakly with the mAChR of rat brain²¹ but little else is known of the action of this toxin in the central nervous system. Preliminary research in the Biochemistry Department at Bath shows the toxin to have sufficient affinity and specificity for the neuronal nAChR to serve as an effective ligand for affinity chromatography, thus providing a means of isolating the neuronal nAChR protein. Then, using the powerful techniques of molecular genetics, the primary sequence data of the receptor protein could be determined. This would allow a full comparison with the peripheral nAChR and permit function to be related to molecular structure.

Over recent years, the principles of affinity ligand chromatography have become well established and this methodology has been applied to a range of biological and chemical problems³⁰. Due to the semi-rigid nature of anatoxin-a, it should be possible to modify its structure for coupling to a solid support without significant loss of potency. The basic structure of a polymer-bound affinity ligand, incorporating anatoxin-a, is shown in (scheme 3). The covalent link will probably be an amide bond as the methodology for forming this bond is well established³⁰.

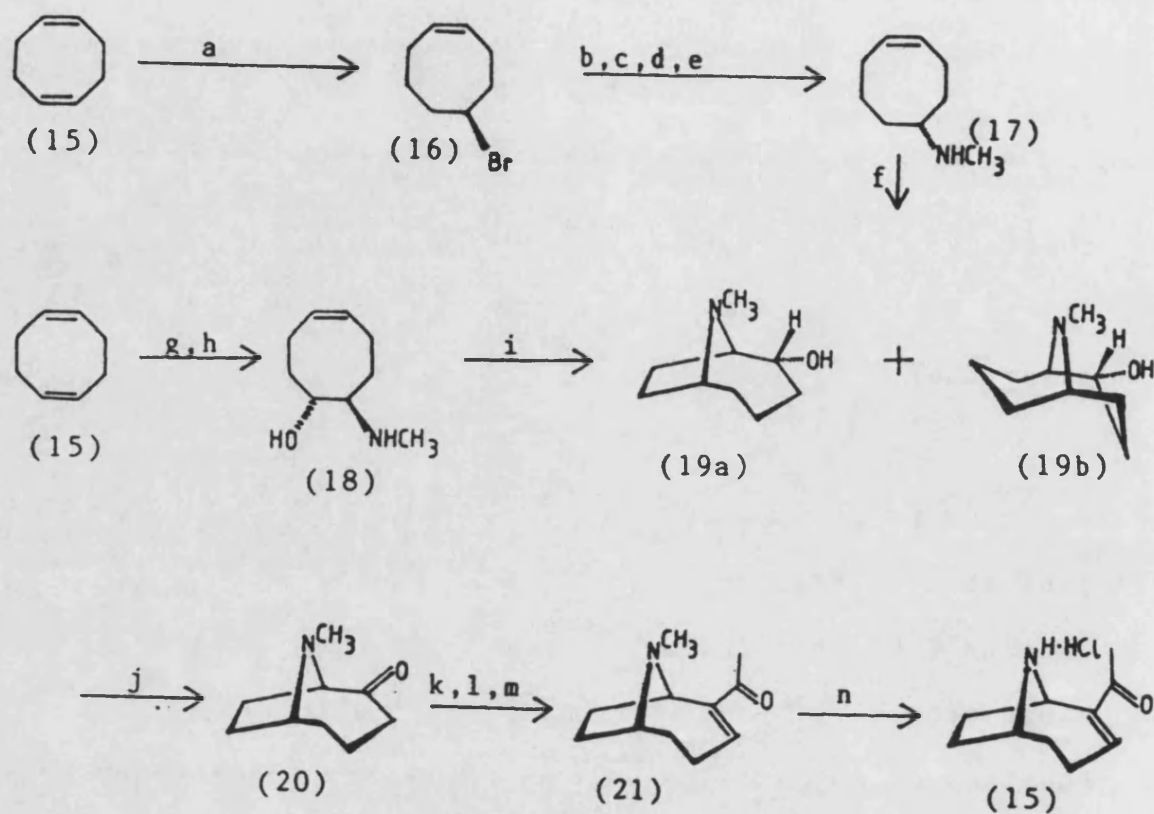


a) conc HCl, reflux, 94%; b) CH_3OH , HCl, 67%;
 c) LiOH, 5% aq CH_3OH , 100%; d) CH_3Li , DME, 76%;
 e) DMSO, NaH, $(\text{CH}_3)_3\text{S(O)I}$, 46%; f) (i) $(\text{C}_2\text{H}_5)_2\text{O}$,
 HCl, (ii) H_2O , hv, 46%; g) benzene, DEAD, 29%; h)
 $(\text{C}_2\text{H}_5)_2\text{O}$, HCl, 100%.

SCHEME 4

Due to the difficulty in isolating natural anatoxin-a, a robust synthetic route is required to produce the toxin and relevant analogues chirospecifically if it is to be used to study the nAChR in neurones. Since the structure of anatoxin-a was first elucidated this alkaloid has aroused the interest of many organic chemists. As a result, a number of successful syntheses of the toxin, both as the racemate³¹⁻³⁹ and as the pure enantiomer,^{40,41,42} have been published.

The first synthetic route to (+)-anatoxin-a was published by Edwards in 1977⁴⁰ (scheme 4). He converted cocaine (10) into the α,β -unsaturated ester (11) by a known procedure⁴³. Hydrolysis of (11) followed by reaction of the lithium salt of the resulting acid with methyl lithium gave the α,β -unsaturated methyl ketone (12). Ring expansion of (12) to the 9-azabicyclo-[4.2.1]non-2-ene ring system was achieved in two steps. First, (12) was treated with sodium dimethyloxosulphonium methylide to give a 2:1 mixture of the endo- (13) to the exo-cyclopropane (14). This proved a problem as (13) and (14) were difficult to separate, which was necessary as only (13) underwent photolytic ring opening to give N-methylanatoxin-a. Finally oxidative cleavage of the N-methyl group gave the natural product as its hydrochloride salt (15) in 2.9% overall yield from cocaine.



a) 34% $\text{HBr}/\text{CH}_3\text{CO}_2\text{H}$, 76%; b) NaN_3 , DMSO, 100%;
 c) LiAlH_4 , $(\text{C}_2\text{H}_5)_2\text{O}$, 72%; d) $\text{ClCO}_2\text{C}_2\text{H}_5$, $(\text{C}_2\text{H}_5)_2\text{O}$, 94%; e) LiAlH_4 , $(\text{C}_2\text{H}_5)_2\text{O}$, 71%; f) (i) HOBBr , (ii) Base, 82%; g) $\text{CH}_3\text{CO}_3\text{H}$, $\text{pHNO}_2\text{C}_6\text{H}_4\text{CO}_3\text{H}$, CHCl_3 , 48%; h) CH_3NH_2 , 75%; i) (i) $\text{Hg}(\text{CH}_3\text{CO}_2)_2$, (ii) NaBH_4 , 95%; j) (i) 3M H_2SO_3 , acetone, (ii) $\text{C}_2\text{H}_5\text{CO}_2\text{H}$, Jones reagent, 44%; k) $n\text{-BuLi}$, $\text{CH}_3\text{CHS(O)Ph}$, THF, 90%; l) $t\text{-BuOK}$, THF, 90%; m) toluene, reflux, 39%; n) (i) DEAD , benzene, Δ , (ii) 1M HCl , 50% aq $\text{C}_2\text{H}_5\text{OH}$, 35%.

SCHEME 5

Although this route is chirospecific it is not one of the better methods for producing large amounts of the toxin. Being only a partial synthesis and having a low overall yield make this route unattractive. The real importance of this synthesis was that it defined the absolute stereochemistry of anatoxin-a. As the absolute stereochemistry of cocaine⁴⁴ was known, so was that of the product of this synthetic route. As the toxicity, rotation and other physical data were identical for synthetic and natural anatoxin-a it was concluded that the absolute stereochemistry was the same.

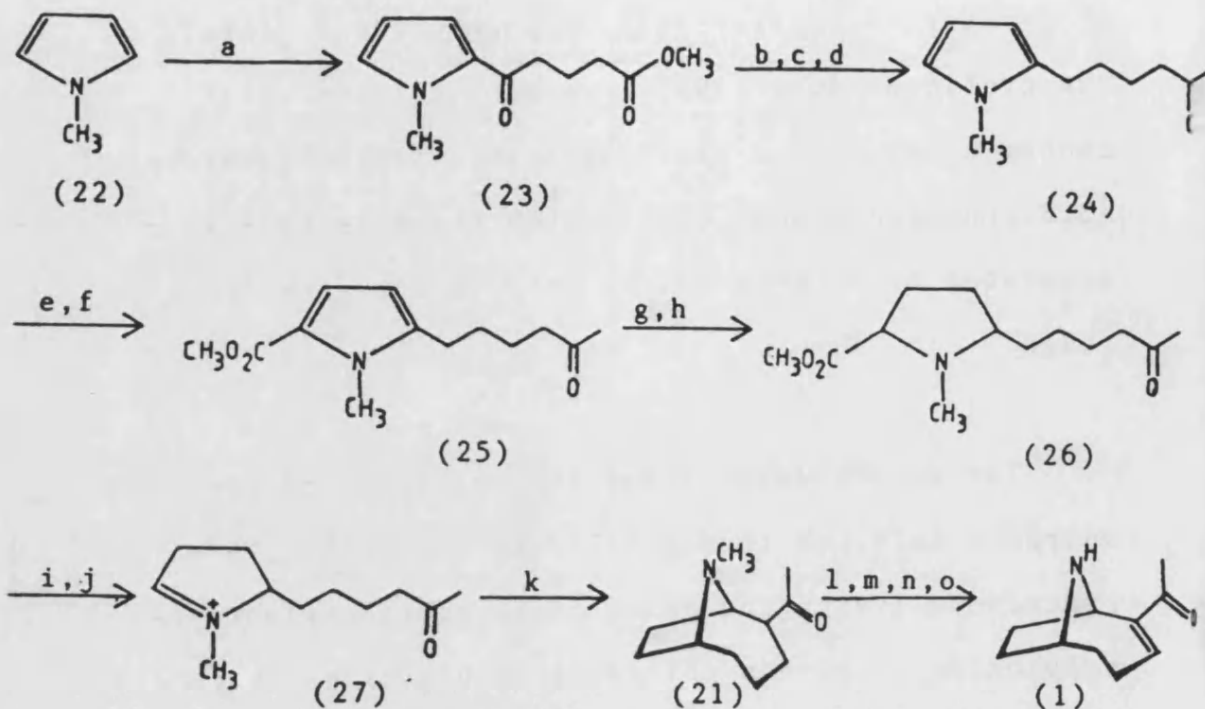
Edwards and co-workers also produced the first total synthesis of (\pm)-anatoxin-a (scheme 5)³¹. They employed two methods that had been developed at that time to synthesise the 9-azabicyclo[4.2.1]nonane ring system. In the first 5-bromocyclooct-1-ene (16) was prepared by the method of Ziegler from 1,5-cyclooctadiene (15)⁴⁵. Bromide (16) was then converted in four steps to 5-methylaminocyclooctene (17). Treatment of (17) with first hypobromous acid and then base gave an epimeric mixture of the bicyclic alcohol (19a)⁴⁶. The second method was that of Barrelle⁴⁷ in which 1,5-cyclooctadiene (15) was converted to its monoepoxide and then to trans-5-hydroxy-6-methylaminocyclooct-1-ene (18). Cyclisation of (18) with mercuric acetate to

give, after borohydride demercuration, a single epimer of 9-methyl-9-azabicyclo[4.2.1]nonan-2 α -ol (19a). The cyclic product (19a) from both methods was contaminated with a small amount of the 9-azabicyclo[3.3.1]nonane isomer (19b) which could be easily separated from the required product after the next stage.

The aminoalcohol (19a) was oxidised, as its sulphate salt, to the bicyclic ketone (20). This reacted well with the anion of α -chloroethylphenylsulphoxide to give a chlorohydrinsulphoxide which upon treatment with base followed by thermal rearrangement gave N-methylanatoxin-a (21).

All that remained was to remove the N-methyl group which was achieved by the same oxidative cleavage as in Edwards' previous synthesis of the toxin. This final step went in a yield of 35% which was an improvement on their earlier effort but still disappointing. The overall yield of the synthetic route from 1,5-cyclooctadiene (15) to (\pm)-anatoxin-a (1) was 1.9%.

Rapoport successfully synthesised (\pm)-anatoxin-a³² via the intramolecular cyclisation between an iminium salt and a carbon atom bearing an electron withdrawing substituent (scheme 6). To do this the correctly

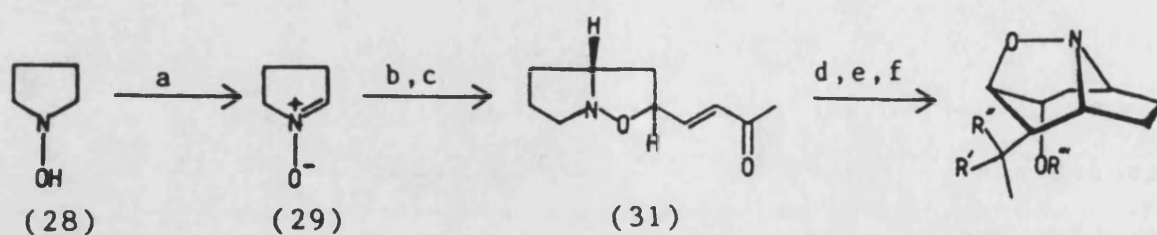


a) AlCl_3 , $\text{ClCO}(\text{CH}_2)_3\text{CO}_2\text{CH}_3$, CH_2Cl_2 , 51%; b) NH_2NH_2 , KOH , ethylene glycol, 100%; c) LiOH , H_2O , 97%;
 d) CH_3Li , THF, 75%; e) Cl_3CCOCl , $(\text{C}_2\text{H}_5)_2\text{O}$, 71%;
 f) NaOCH_3 , CH_3OH , 70%; g) Rh , alumina, H^+ , CH_3OH , 78%; h) CrO_3 , H_2SO_4 , acetone, 88%; i) 6M HCl , 100%;
 j) POCl_3 , 100%; k) CH_3OH , HCl , 49%; l) (i) $(\text{CH}_3\text{-CO})_2\text{O}$, (ii) HBr , 95% m) Br_2 , CHCl_3 , 43%; n) LiBr , 40%; o) benzene, DEAD, 29%.

SCHEME 6

substituted pyrrolidine had to be obtained. This was achieved by first acylating 1-methylpyrrole (22) with 4-methoxycarbonylbutanoyl chloride under Friedel-Crafts conditions to give the 2-substituted pyrrole (23). A problem encountered at this stage was the formation of a significant amount of the 3-substituted pyrrole. The unusual abundance of this normally rare positional isomer was evidently a result of the steric bulk of the entering glutarate moiety, however the two isomers were easily separated by distillation. Pyrrole (23) was converted to the methylketone (24) in three standard steps and this was then acylated with trichloroacetyl chloride to give, following treatment with sodium methoxide, the disubstituted pyrrole (25). This pyrrole was reduced catalytically to the corresponding 2,5-disubstituted methylpyrrolidine, however, Jones oxidation was required to restore the reduced ketone functionality.

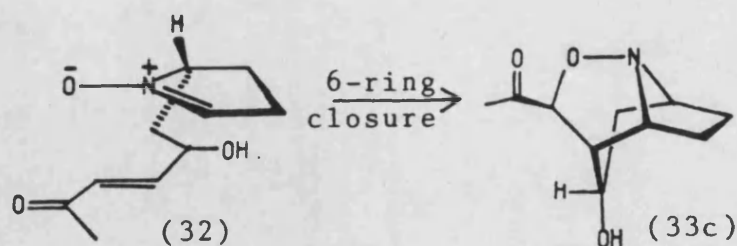
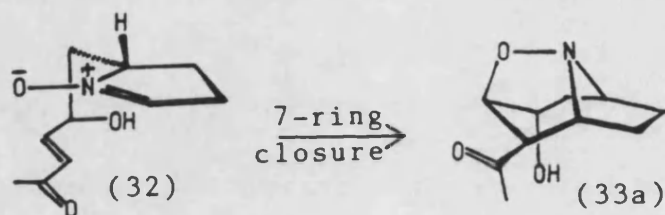
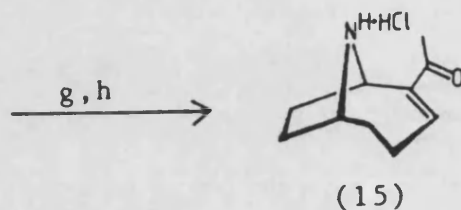
With the correctly substituted pyrrolidine (26) in hand, acid catalysed ester hydrolysis followed by phosphorus oxychloride-induced decarbonylation generated the required iminium ion (27) which underwent thermal cyclisation to give N-methyldihydroanatoxin-a (21). This constituted a formal synthesis of the toxin as Edwards had converted (21) to anatoxin-a in four steps and 4.7% yield. A problem with the cyclisation reaction is that it is reversible but under



(33 a) $R', R'' = O; R''' = H$

(33 b) $R' = OCH_2CH_2O;$

$R''' = SO_2CH_3$



a) HgO , benzene, 100%; b) $CH_2:CHCH:CHCH(OH)CH_3$ (30), benzene, reflux, 70%; c) MnO_2 , celite, CH_2Cl_2 , 96%; d) MCPBA, CH_2Cl_2 , 71%; e) ethylene glycol, PTSA, benzene, 96%; f) CH_3SO_2Cl , $(C_2H_5)_3N$, CH_2Cl_2 , 94%; g) (i) $LiAlH_4$, $NiCl_2$, THF, $-40^\circ C$, (ii) PTSA, acetone, (iii) $NaHCO_3$, H_2O , (iv) $(t-BuO_2C)_2O$, $CHCl_3$ 42.5%; h) 3M HCl, EtOAc, 100%.

SCHEME 7

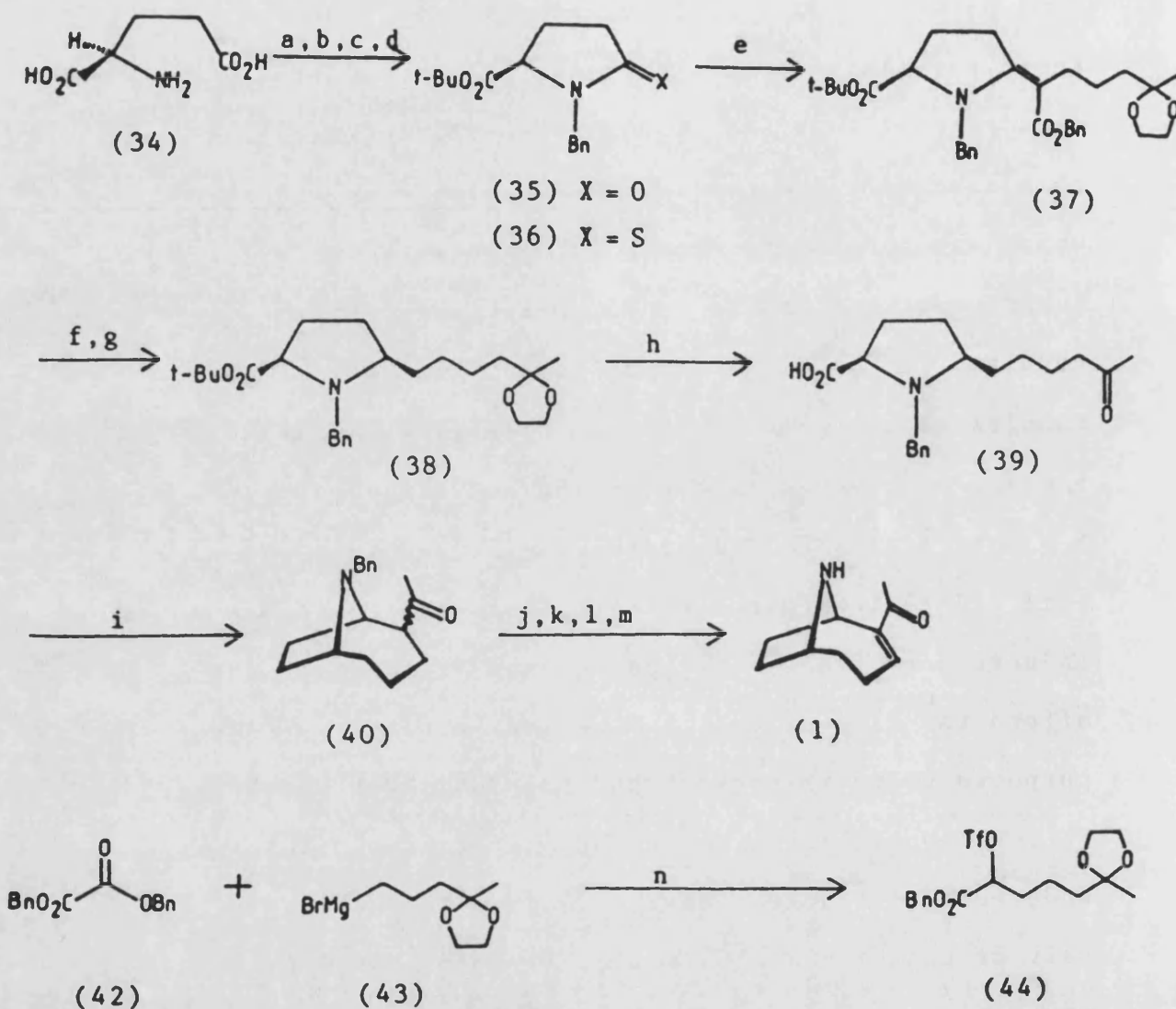
these conditions the iminium ion also undergoes an irreversible polymerisation reaction which resulted in drastically reduced yields of (21). Thus the reaction conditions have to be finely balanced to give the best yield of (21), which is only 49%.

The overall yield of anatoxin-a from N-methylpyrrole by this method is a poor 0.3%. Problems can also be foreseen if attempts to prepare substituted analogues of the natural product were made. Increased steric hindrance may cause an increase in the formation of the 3-substituted pyrrole in the first acylation reaction, it may also cause a decrease in the rate of cyclisation of the iminium ion probably resulting in an increase in the polymerisation side reaction.

Tufariello has reported an efficient nitron-based synthesis of (+)-anatoxin-a (scheme 7)^{33,34}. Treatment of 1-hydroxypyrrolidine (28) with mercuric oxide generated 1-pyrroline-1-oxide (29). This nitron underwent 1,3-dipolar cycloaddition reaction with diene (30) to afford an isoxazolidine which when oxidised with manganese dioxide gave the ketone (31). Oxidative cleavage of the isoxazolidine ring of (31) produced the nitron (32). Upon warming (32) gave the single cycloadduct (33a) which possessed the required 9-azabicyclo[4.2.1]nonane ring system. Nitrones are

known to undergo closure with the new carbon-carbon bond being formed at the least hindered carbon of the recipient alkene. Thus nitron (32) can undergo closure to give either a 6- or 7-membered ring. In this case only the required 7-membered ring was formed, which was consistent with previous observations⁴⁸. Subsequent ketalisation and mesylation of (33a) resulted in the formation of (33b). The action of a complex reducing agent on (33b) resulted in both removal of the mesylate function and cleavage of the N-O bond of the isoxazolidine. p-Toluenesulphonic acid (PTSA) treatment of the hydroxy ketal product induced both trans ketalisation and dehydration to afford the PTSA salt of the natural product. For the purposes of purification the crude salt was converted to its t-butylcarbamate (t-BOC) derivative. This underwent mild acid cleavage to give the hydrochloride salt of anatoxin-a (15) in 18.3% yield from 1-hydroxy-pyrrolidine.

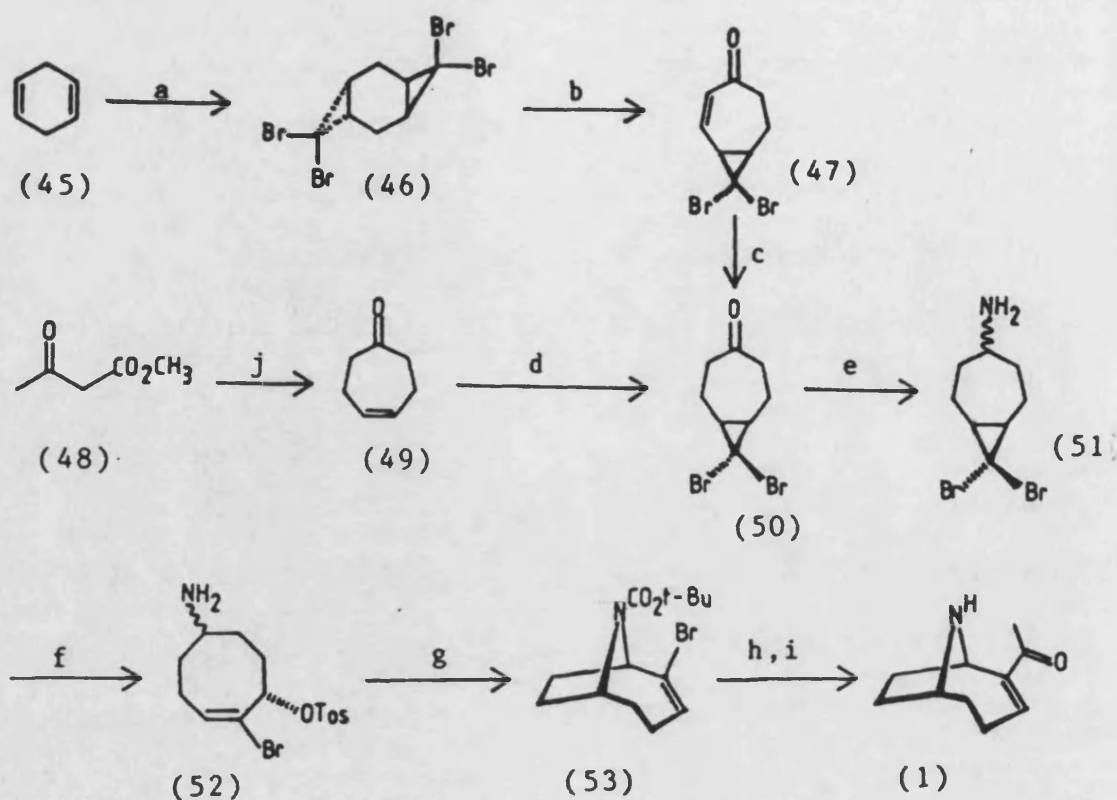
Rapoport and Koskinen reported a chirospecific synthesis of anatoxin-a in 1985⁴² (scheme 8). It was essentially an optimised version of the process first designed by the same group in 1984⁴¹, where both the overall yield and the enantiomeric purity of the final product were improved. First t-butyl N-benzylpyroglutamate (35) was prepared from optically pure



a) (i) NaOH, benzaldehyde, (ii) NaBH₄, (iii) H⁺, 97%;
 b) heat, 80%; c) (i) ClCOCOC₂H₅, (ii) t-BuOH, 93%; d) P₄-S₁₀, 87%; e) (i) triflate (44), CH₃CN, (ii) PPh₃, CH₂Cl₂, 84%; f) Pd/C, NH₄(O₂CH), CH₃OH, 67%; g) benzyl bromide, K₂CO₃, CH₃CN; h) CH₃CO₂H, i-PrOH, H₂O, 100%; i) (i) POCl₃, (ii) KCN aq., CH₂Cl₂, (iii) CH₃OH, HCl, 70%; j) (i) CH₃OH, Pd/C, H₂, H⁺, (ii) (t-BuO₂C)₂O, CH₃OH, 70%; k) (i) KH, THF, (ii) TMSCl, (C₂H₅)₃N, 100%; l) Pd(O₂CCH₃)₂, (C₂H₅)₃N, CH₃CN, 52%; m) CF₃CO₂H, 97%; n) (i) Pd/C, H₂, EtOAc, (ii) (CF₃SO₂)₂O, CH₂Cl₂, 55%.

SCHEME 8

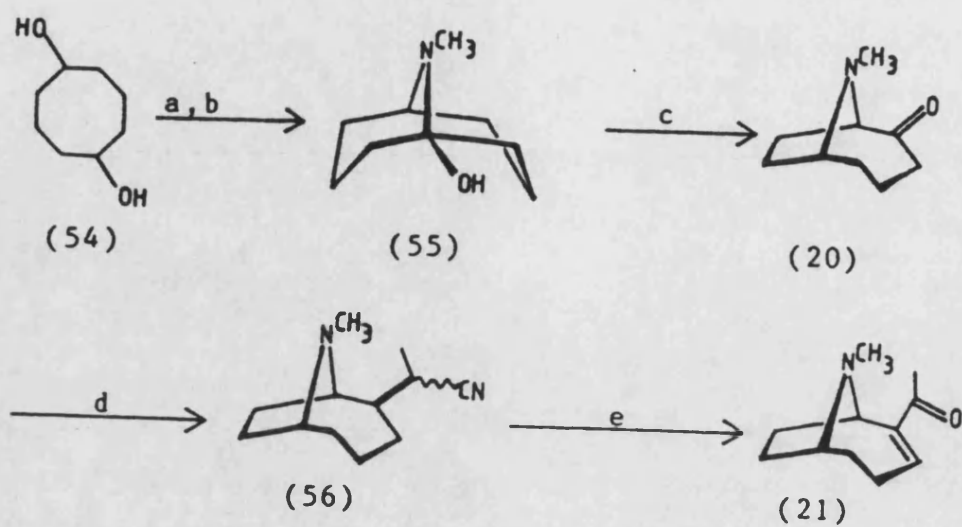
glutamic acid (34)⁴¹. This was then converted to the thiolactam (36) which was alkylated with the triflate (44). Triphenylphosphine-induced sulphide contracture of the S-alkylated product led to the vinyligous carbamate (37). The sulphide contracture^{ion} reaction presented a problem as the basic conditions required resulted in racemisation of the product. The reaction was found to be very dependant on both pH and temperature, but when the conditions were monitored carefully good yields of product could be obtained in high enatiomeric purity. Reduction of (37) followed by rebenzylation gave the amine (38) and succeeded in setting the stereochemistry at the 5-position of the pyrrolidine ring, although it should be noted that a small amount of the trans-isomer was also formed. Acid hydrolysis of (38) gave the pyrrolidine (39) which bore the correct substituents for iminium ion formation and cyclisation. Indeed, treatment of (39) with phosphorus oxychloride gave an iminium ion, which when first trapped with potassium cyanide, cyclised under acid conditions to give N-benzyl dihydroanatoxin-a (40). This N-benzyl derivative (40) could be converted to the natural product (1) in 5 steps and 35% yield. The overall yield of optically pure anatoxin-a (1) from glutamic acid (34) was 8.1%.



a) CHBr_3 , NaOH , CH_2Cl_2 , 77%; b) $\text{Ag}(\text{O}_2\text{CCF}_3)$, conc H_2SO_4 , CH_2Cl_2 , 29%; c) $(\text{PPh}_3)_3\text{RhCl}$, H_2 , benzene, 99%;
 d) $\text{C}_6\text{H}_5\text{HgCBr}_3$, benzene, 71%; e) NaBH_3CN , ammonium acetate, i-propanol, 94%; f) (i) PTSA, benzene, (ii) silver tosylate, CH_3CN ; g) (i) benzene, HBr , $h\nu$, (ii) $(\text{C}_2\text{H}_5)_3\text{N}$, CH_3CN , 70°C , (iii) $(t\text{-BuO}_2\text{C})_2\text{O}$, CH_2Cl_2 , 32%; h) $t\text{-BuLi}$, $\text{CH}_3\text{C}(\text{O})\text{N}(\text{CH}_3)\text{OCH}_3$, THF, 73%; i) $\text{CF}_3\text{-CO}_2\text{H}$, CH_2Cl_2 , 98%; j) (i) NaH , (ii) $n\text{-BuLi}$, (iii) $\text{ClCH}_2\text{-CH=CHCH}_2\text{Cl}$, 30%; k) NaOCH_3 , 60%; l) LiI , 67%.

SCHEME 9

Danheiser produced another synthetic route to racemic anatoxin-a (1) (scheme 9)³⁵. An important intermediate of this route was 8,8-dibromobicyclo[5.1.0]octan-4-one (50), which he obtained by two approaches. The first employed the action of the Seyferth reagent on 4-cycloheptenone (49) to give the bicyclic ketone (50) directly. However, they turned to an alternative route for the production of large quantities of material. This involved forming the tetrabromotricyclooctane (46) from cyclohexadiene (45) using the cyclopropanation procedure of Makosza and Fedorynski⁴⁹. Controlled electrocyclic opening of one of the cyclopropane rings in (46) gave the α,β -unsaturated ketone (47) which could be selectively reduced with Wilkinson's catalyst to give the required bicyclic ketone (50). Reductive amination of (50) gave the bicyclic amine (51), which was converted to its acid salt and treated with silver tosylate to effect electrocyclic ring opening to yield (52). It was possible to recover the silver salt thus reducing the financial cost of the reaction. Photoisomerisation and transanular cyclisation of (52) were achieved in a single operation to give the desired 9-azabicyclo[4.2.1]nonane system which was immediately converted to its t-boc derivative (53). Anatoxin-a (1) was then obtained by first treating the vinyl bromide (53) with t-butyl lithium and trapping the resulting vinyl lithium species with N-methoxy-N-methylacetamide. Finally acid



a) CrO_3 , H_2SO_4 , acetone, 49%; b) 40% aq CH_3NH_2 , PTSA, 82%; c) pyridine hydrobromide, Br_2 , $\text{CH}_3\text{CO}_2\text{H}$, 56%; d) NaNH_2 , $(\text{C}_2\text{H}_5\text{O})_2\text{P}(\text{O})\text{CH}(\text{CH}_3)\text{CN}$, THF, 64%; e) (i) THF, lithium diisopropylamide, HMPA, O_2 , (ii) 1M aq Na_2SO_3 , 43%.

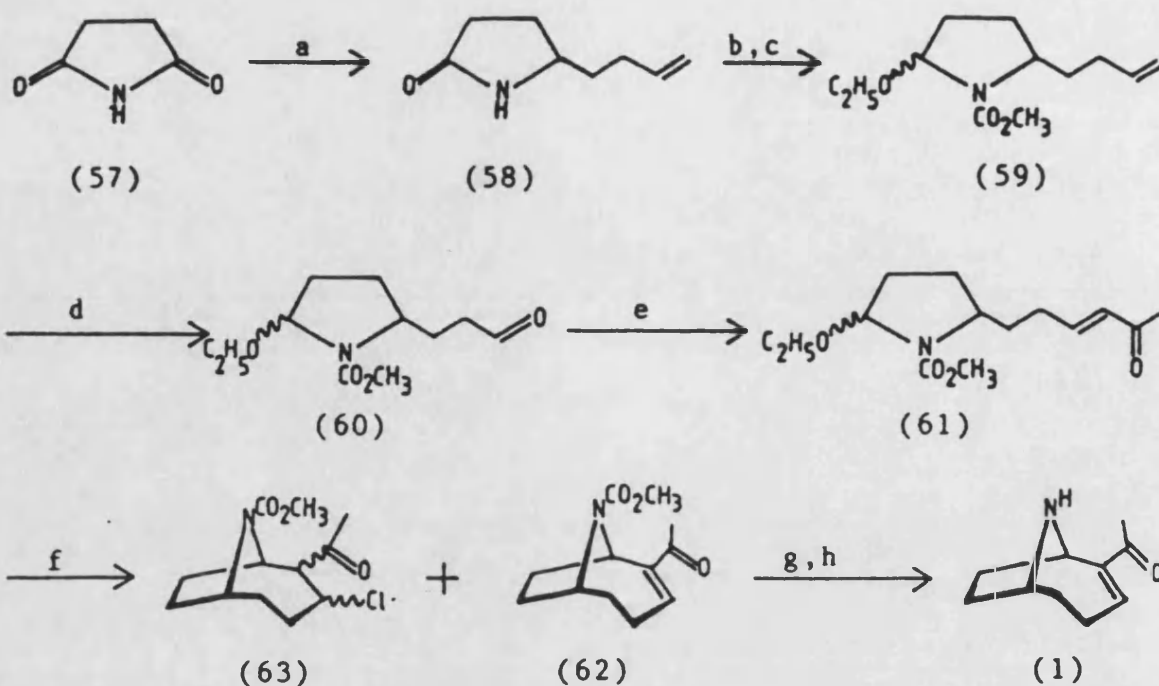
SCHEME 10

hydrolysis removed the Nitrogen protecting group.

The overall yield of the sequence from 1,4-cyclohexadiene (45) to the toxin is 4.8% but from 4-cycloheptenone (49) it is 15.3%. Enone (49) can be prepared in three steps from methyl-3-ketobutanoate (48) in 12% yield.

The next synthesis of racemic anatoxin-a to be published was that of Wiseman and Lee (scheme 10)³⁶. Theirs was a short synthesis, the key feature of which was the development of a new method for the construction of the 9-azabicyclo[4.2.1]nonane ring system. This was accomplished by the reorganisation of 9-methyl-9-azabicyclo[3.3.1]nonan-1-ol (55). The amino alcohol (55) was easily accessible in two steps from 1,5-cyclooct-^{ne}adiol. Bromination of (55) gave the [4.2.1] amino ketone (20) directly, presumably through bromination and cyclisation of 5-(methylamino)cyclooctanone.

The method of Wroble and Watt⁵⁰ for bishomologation of ketones into α,β -unsaturated ketones was used for the conversion of amino ketone (20) into N-methyl-anatoxin-a (21). Reaction of (20) with diethyl-(cyanoethyl)phosphonate gave (56) as a mixture of isomers. Deprotonation of (56) afforded a delocalised anion which could be trapped by oxygen at the



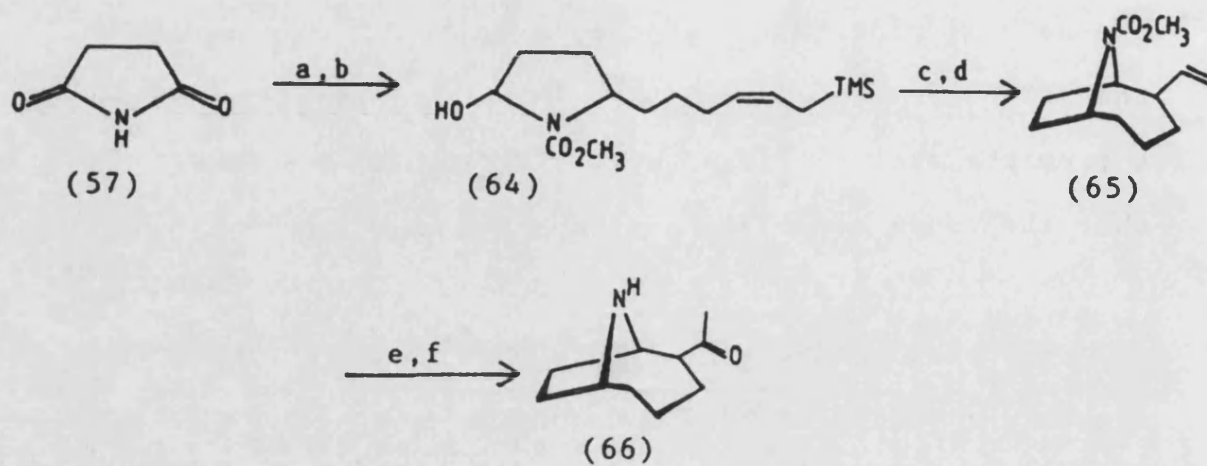
a) (i) CH_3MgCl , THF, (ii) $\text{CH}_2=\text{CHCH}_2\text{CH}_2\text{MgBr}$, THF, (iii) NaBH_3CN , HCl , THF, 53%; b) (i) lithium diisopropylamide, THF, (ii) CH_3COCN , THF, 69%; c) (i) NaBH_4 , $\text{C}_2\text{H}_5\text{OH}$, (ii) H_2SO_4 , $\text{C}_2\text{H}_5\text{OH}$, 77%; d) (i) O_3 , CH_2Cl_2 , (ii) $(\text{CH}_3)_2\text{S}$, CH_2Cl_2 , 75%; e) dimethyl(2-oxopropyl) phosphonate, $i\text{-Pr}_2\text{NC}_2\text{H}_5$, LiCl , CH_3CN , 83%; f) HCl , CH_3OH , 58%; g) DBM, toluene, 60%; h) TMSI , CH_3CN , 55%.

SCHEME 11

α -position. The hydroperoxide produced was reduced and hydrolysis of the resulting cyanohydrin gave N-methylanatoxin-a (21). Deprotection of (21) to give the natural product (1) was achieved in 35% yield with the method developed by Edwards³¹. The yield of anatoxin-a from the diol (54) was only 2.2% despite the fact that the route comprises only six steps.

Speckamp and co-workers produced an approach to anatoxin-a (scheme 11)³⁷ which bore a good deal of resemblance to Rapoport's synthesis in which the key step is the Mannich-type cyclisation of an iminium ion. The main difference in the two routes was Speckamp's use of a carbomethoxy protecting group on nitrogen. This enhanced the electrophilicity of the iminium ion as well as reducing the number of protection and deprotection steps required. They also succeeded in using an α,β -unsaturated ketone as a nucleophile, thus leading, in a more direct way, to the desired enone.

Speckamp's synthesis began with the conversion of succinimide (57) into butenylpyrrolidone (58) in a one pot reaction using known chemistry^{51,52}. Pyrrolidone (58) was protected as its methylcarbamate, reduction of which followed by in situ ethanolysis gave ethoxy carbamate (59). Ozonolysis of (59) gave aldehyde (60) after dimethyl sulphide reduction. Enone (61) was



a) (i) CH_3MgCl , THF, (ii) $\text{TMSCH}_2\text{CH}=\text{CH}(\text{CH}_2)_3\text{MgBr}$, THF, (iii) NaBH_3CN , HCl , THF, 60%; b) (i) lithium diisopropylamide, THF (ii) CH_3OCCN , THF, 90%; c) NaBH_4 , HCl , $\text{C}_2\text{H}_5\text{OH}$, 100%; d) $\text{H}_2\text{CO}_2\text{H}$, 73%; e) CuCl_2 , PdCl_2 , O_2 , DMF, 64%; f) TMSI , CH_3CN , 90%.

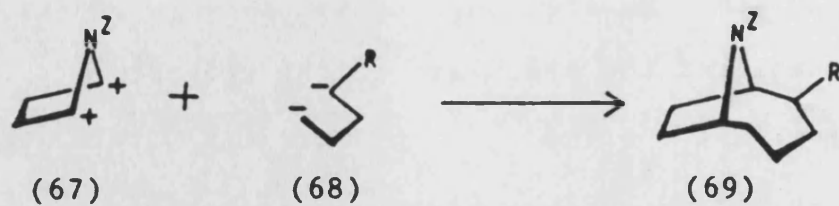
SCHEME 12

prepared by the reaction of (60) with dimethyl(2-oxopropyl)phosphonate under Masamune-Roush conditions. This completed the synthesis of the precursor for the key cyclisation step.

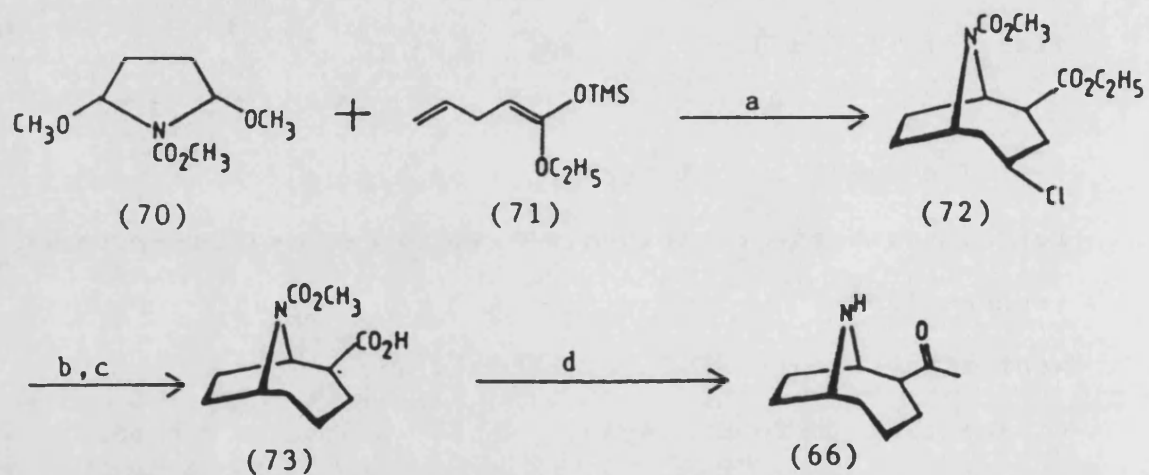
When (61) was treated with methanolic HCl it gave an inseparable mixture of the enone (62) and the stereoisomeric chlorides (63). Dehydrochlorination of (63) with DBN gave pure (62). The synthesis of anatoxin-a (1) was completed through the deprotection of nitrogen with trimethylsilyl iodide. The overall yield of (1) is 3.4% from succinimide.

The same group later produced a more efficient formal synthesis of anatoxin-a (1) by a similar approach (scheme 12)³⁸. The hydroxycarbamate (64) readily underwent ring closure to give the bicyclic olefin (65) by dissolution in formic acid. Wacker oxidation of (65) followed by deprotection of nitrogen gave dihydro-anatoxin-a (66) which had been converted by earlier workers to anatoxin in four steps and 50.4% yield⁴¹. This gave an overall yield to the toxin from succinimide of 11.5%.

The latest synthesis to be reported is that of Shono and co-workers. Their strategy involved a one step construction of the 9-azabicyclo[4.2.1]nonane ring skeleton (69) by the reaction of a dication (67)



SCHEME 13



a) TiCl_4 , 60%; b) H_2 , Ra-Ni , KOH , 95%; c) KOH , 100%; d) (i) CH_3Li , (ii) hydrolysis, 40%.

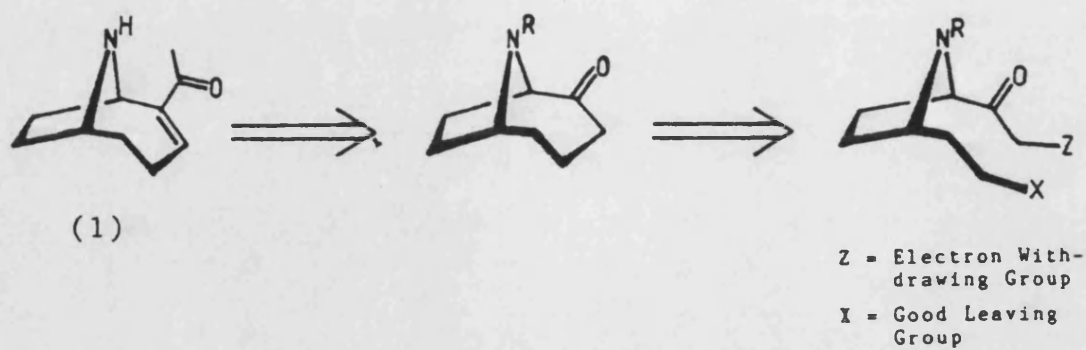
SCHEME 14

with a dianion (68) (scheme 13).

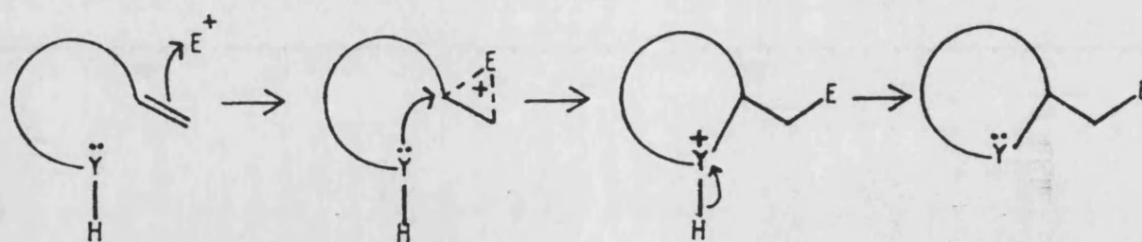
Titanium tetrachloride treatment of pyrrolidine (70) formed an active species equivalent to (67). This could be trapped with diene (71), acting as an equivalent of (68), to give the 9-azabicyclo [4.2.1]-nonane (72). Hydrogenolysis of (72) then hydrolysis of the dechlorinated product yielded the carboxylic acid (73). Subsequent treatment of (73) with an excess of methyl lithium, followed by hydrolysis, afforded dihydro-anatoxin-a (66) which Rapoport had converted to pure anatoxin-a (1) in 50.4% yield⁴¹. The overall yield of the synthesis from (70) to anatoxin-a (1) was 11.5%.

The most efficient process of those discussed was the one designed by Tufariello (scheme 7)³⁴ with an overall yield of 18.3%. The routes of Danheiser (scheme 9)³⁵, Speckamp (scheme 12)³⁸ and Shono (scheme 14)³⁹ also gave respectable overall yields of greater than 10%. Thus there are a good range of methods available for producing racemic anatoxin-a. These routes could presumably be developed to give many analogues of anatoxin-a as well. However, although Rapoport's chirospecific route gives pure natural or unnatural anatoxin-a in a respectable 8% overall yield, it is not ideal for the synthesis of optically pure anatoxin-a analogues. Thus our aim was to develop a

new route to anatoxin-a which could be readily adapted to give the toxin and analogues of (1) chirospecifically.



SCHEME 15



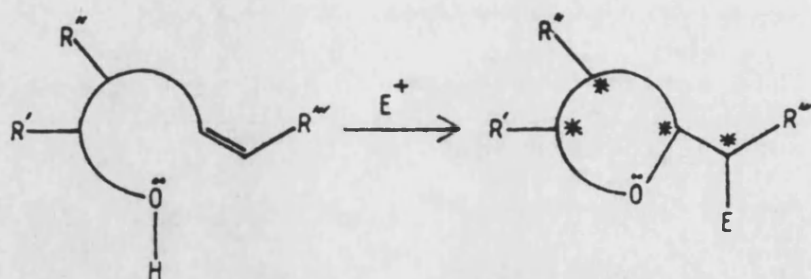
SCHEME 16

RESULTS AND DISCUSSION

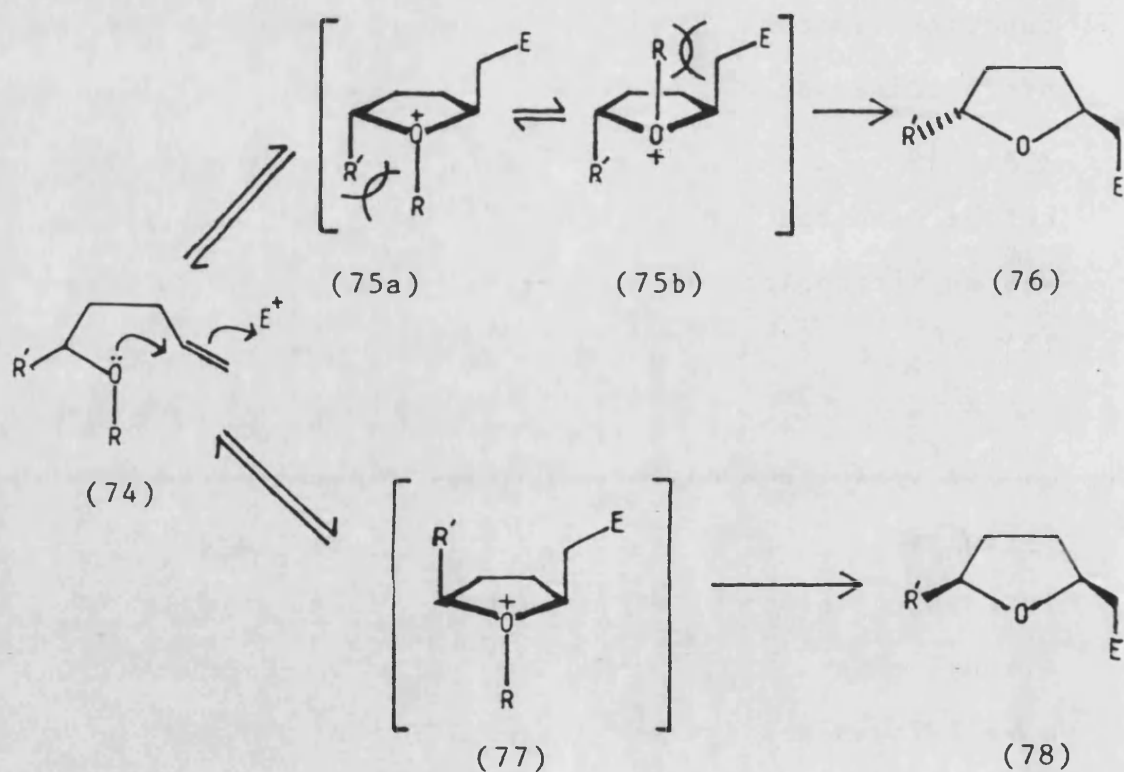
The aim of this project was to develop a new chirospecific route to the neurotoxic alkaloid anatoxin-a. This natural product's unusual ring structure and profound neurotoxic activity has invoked considerable interest from synthetic organic chemists. All these aspects have been discussed earlier.

A retro-synthetic analysis of the problem at hand suggests that anatoxin-a may be synthesised from a suitably functionalised disubstituted pyrrolidine. The functionalisation of the substituents present in such a pyrrolidine would have to allow cyclisation to give the 9-azabicyclo[4.2.1]nonane ring system and subsequent ketone homologation. The mode of cyclisation planned was an intramolecular alkylation, as shown in (scheme 15).

A useful method for preparing disubstituted pyrrolidines is the electrophilically initiated intramolecular addition of suitably substituted γ -alkenyl amines⁵³. Electrophilic addition reactions are a well known and extremely useful method for adding substituents across a double bond⁵⁴. The general way in which these reactions are thought to proceed when forming heterocycles is shown in (scheme 16).



SCHEME 17



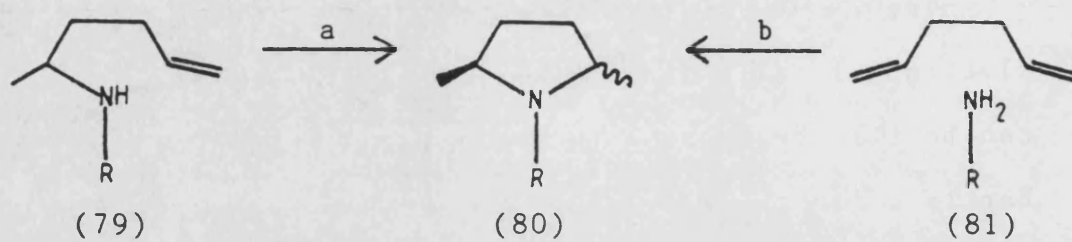
(74)	R	R ¹	Cis/Trans	Yield%
a	H	CH ₃	0.5	66
b	CH ₃	CH ₃	0.5	15
c	CH ₂ C ₆ H ₅	CH ₃	2	60
d	DCB	CH ₃	21	63

Reaction conditions: I₂, CH₃CN, 0°C (NaHCO₃ included for 74a)

SCHEME 19

It is presumed that the addition takes place by the initial formation of a complex between the olefin and the electrophile. This complex is then thought to collapse by the addition of some nucleophile⁵⁴.

The formation of complex heterocycles by electrophilically induced intramolecular cyclisation can be illustrated by the strategy developed by Bartlett for the synthesis of cyclic polyethers (scheme 17)^{55,56}. A qualitative understanding of the factors governing the stereochemical outcome of these cyclisations was gained by studying a variety of derivatives of 5-hexen-2-ol (74) (scheme 19)⁵⁶. It was deduced that loss of 'R' from the oxonium ion intermediates (75) and (77) must be slow compared with the reverse reaction (i.e. ring opening), so that (77) will be favoured thermodynamically as well as kinetically. When loss of 'R' is too fast, as in the case of (74c), only a small preference for the cis-product (78) is observed. However, if cleavage of 'R' is too slow, as for (74b), the yield of the reaction is cut significantly due to the increased prominence of competing side reactions. 'R' must also be sufficiently bulky to induce a significant steric effect but not so large as to prevent cyclisation. If 'R' is the 2,6-dichlorobenzyl (DCB) group the right balance of electronic and steric properties is found. Thus cyclisation of (74d) results



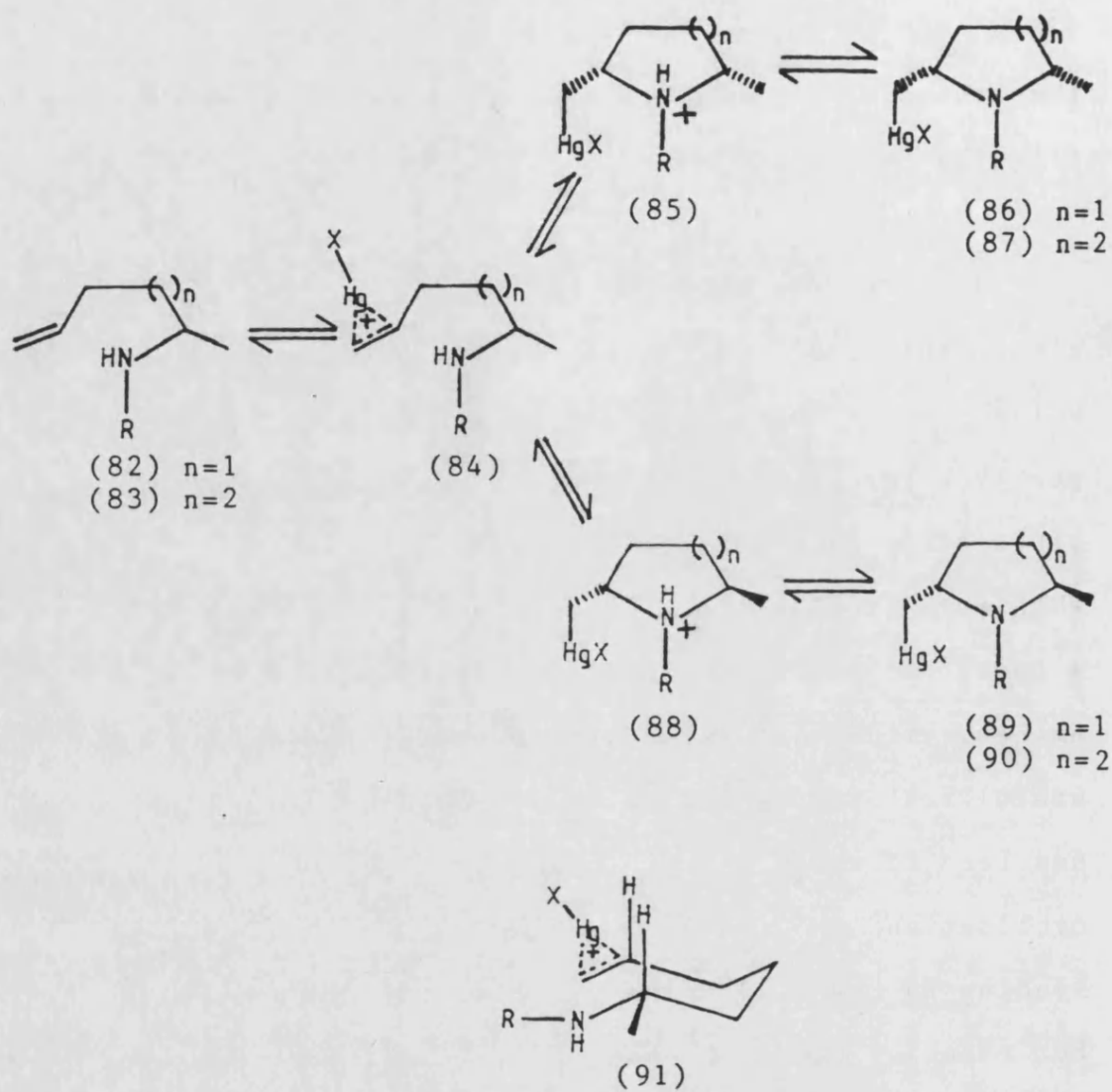
a) reference 61; b) reference 62;

SCHEME 20

in a respectable yield of predominantly the cis-product (78). The mechanism however is probably more complex than this as oxonium ions have been shown to be only slightly pyramidal⁵⁷.

At first not as much work was attempted on the electrophilically initiated cyclisation of alkenyl amines as for the corresponding alcohols. This was partly a result of the conditions employed (e.g. Br₂) often being incompatible with the nitrogen function. When mercury(II)salts were employed as electrophiles a number of elegant methods for cyclising δ -alkenyl amines, amides and carbamates were developed providing useful routes to substituted pyrrolidines⁵⁸. Attention has been focussed on the stereochemistry of the cyclisation⁵⁹ with particular emphasis on the process leading to cis- and trans-2,5-disubstituted pyrrolidines. For example, the synthesis of 2,5-dimethylpyrrolidine (80) from either alkene (79) or hexa-1,5-diene (81) has been achieved and conditions found yielding cis- or trans-(80) selectively (scheme 20).

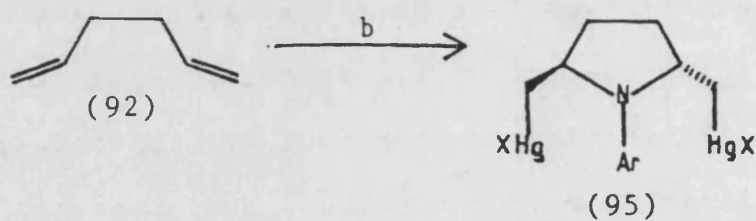
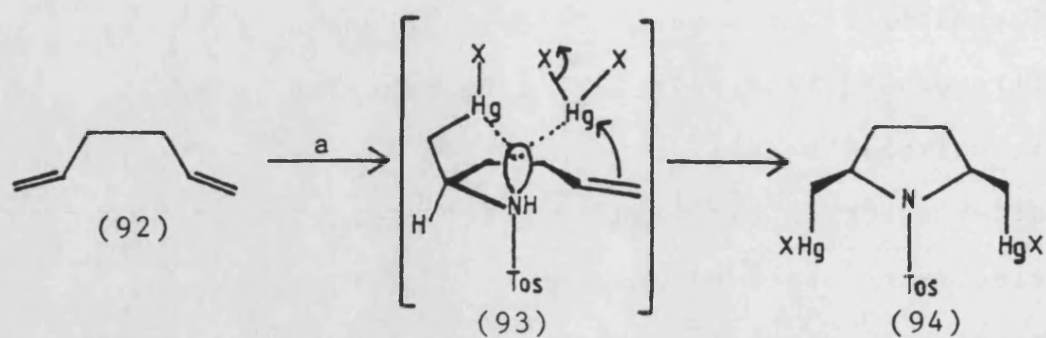
Harding studied the stereoselectivity of the intramolecular amido mercuriation of carbamates (82) and (83)⁶¹. This was shown to be critically dependant on whether the reaction conditions resulted in kinetic or thermodynamic control of the products (scheme 21).



SCHEME 21

Treatment of (83) with $\text{Hg}(\text{O}_2\text{CCH}_3)_2$ in tetrahydrofuran (kinetic conditions) gave a 3:2 mixture of (90) to (87). Alternatively treatment of (83) with $\text{Hg}(\text{O}_2\text{CCF}_3)_2$ in nitromethane (thermodynamic conditions) gave almost exclusively the cis-product (87). A study of this latter reaction by ^1H nmr showed that the stereoselectivity observed was a result of equilibration. Immediately after the addition of the mercuric salt there was a complete loss of starting material and the formation of a 1:1 mixture of (87) and (90). If the reaction was allowed to continue complete conversion of the trans-product (90) to the cis-product (87) was observed. These results show that the initial cyclofunctionalisation reaction of (83) with mercuric ion is nonstereoselective but that, under conditions favouring equilibration, conversion of the trans-product into the cis-product occurs. This may be rationalised in terms of a preference for the intermediacy of a chair conformer (91) in which the substituents adopt equatorial positions.

The form of conformational bias previously discussed does not exist to the same extent in a pyrrolidine ring. If carbamate (82) is treated with $\text{Hg}(\text{O}_2\text{CCH}_3)_2$ in THF (kinetic conditions) the trans-isomer (89) is formed exclusively. However if the reaction is conducted using $\text{Hg}(\text{O}_2\text{CCF}_3)_2$ in nitromethane (thermodynamic conditions) then, although the trans-product



a) $\text{Hg}(\text{NO}_3)_2$, $p\text{-CH}_3\text{C}_6\text{H}_4\text{SO}_2\text{NH}_2$, CH_2Cl_2 ;

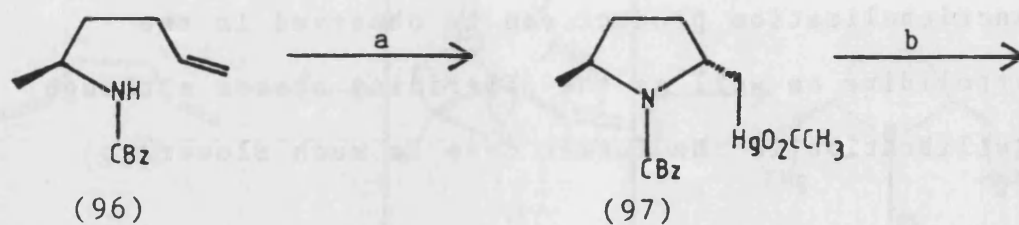
b) $\text{Hg}(\text{O}_2\text{CCH}_3)_2$, ArNH_2 , CH_2Cl_2 .

SCHEME 22

is formed initially, this equilibrates over a period of several days to give a 7:3 mixture in favour of the cis-product. Thus equilibration of the initial cyclo-functionalisation product can be observed in the pyrrolidine as well as the piperidine system although equilibration in the former case is much slower.

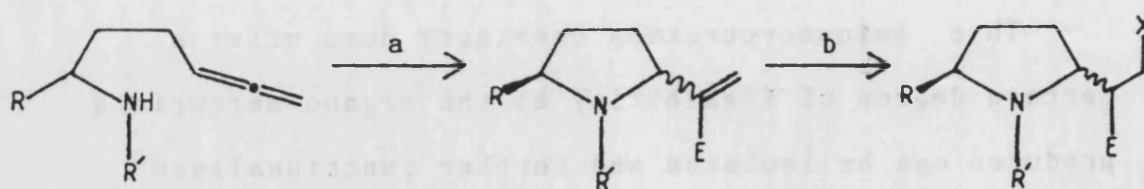
Barluenga reported the cyclisation of a methylene chloride solution of 1,5-hexadiene (92) with p-toluene-sulphonyl amide in the presence of $\text{Hg}(\text{NO}_3)_2$ to give exclusively the cis-adduct (94)⁶². This stereoselectivity was rationalised as being a result of the electron lone pair of the nitrogen atom interacting with and directing the addition of the mercury electrophile, possibly giving rise to the proposed intermediate (93). Little evidence was presented to support the intermediacy of (93) and furthermore, Barluenga observed that the basicity of the nitrogen atom was also important. The aminomercuriation of (92) with aniline derivatives gave predominantly the trans-isomers (95).

This aminomercuriation chemistry does offer a certain degree of flexibility as the organo-mercurials produced can be isolated and further functionalised⁶³. Although the organo-mercury bond resists direct attack by typical carbon electrophiles⁶³, upon suitable activation a variety of such carbon-carbon bond formations may be actuated. The most widely used activation



a) $\text{Hg}(\text{O}_2\text{CCH}_3)_2$; b) NaBH_4 ; c) $\text{CH}_2=\text{CHCO}_2\text{CH}_3$.

SCHEME 23

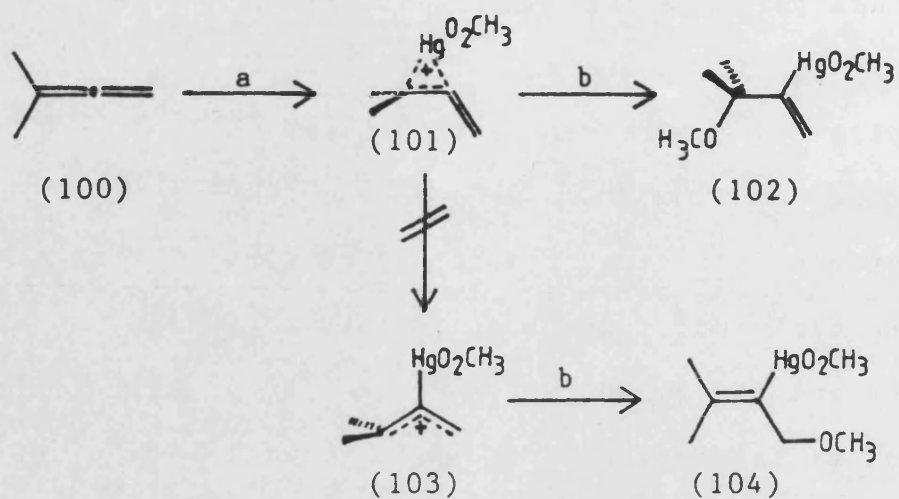


a) E^+ , b) HX

SCHEME 24

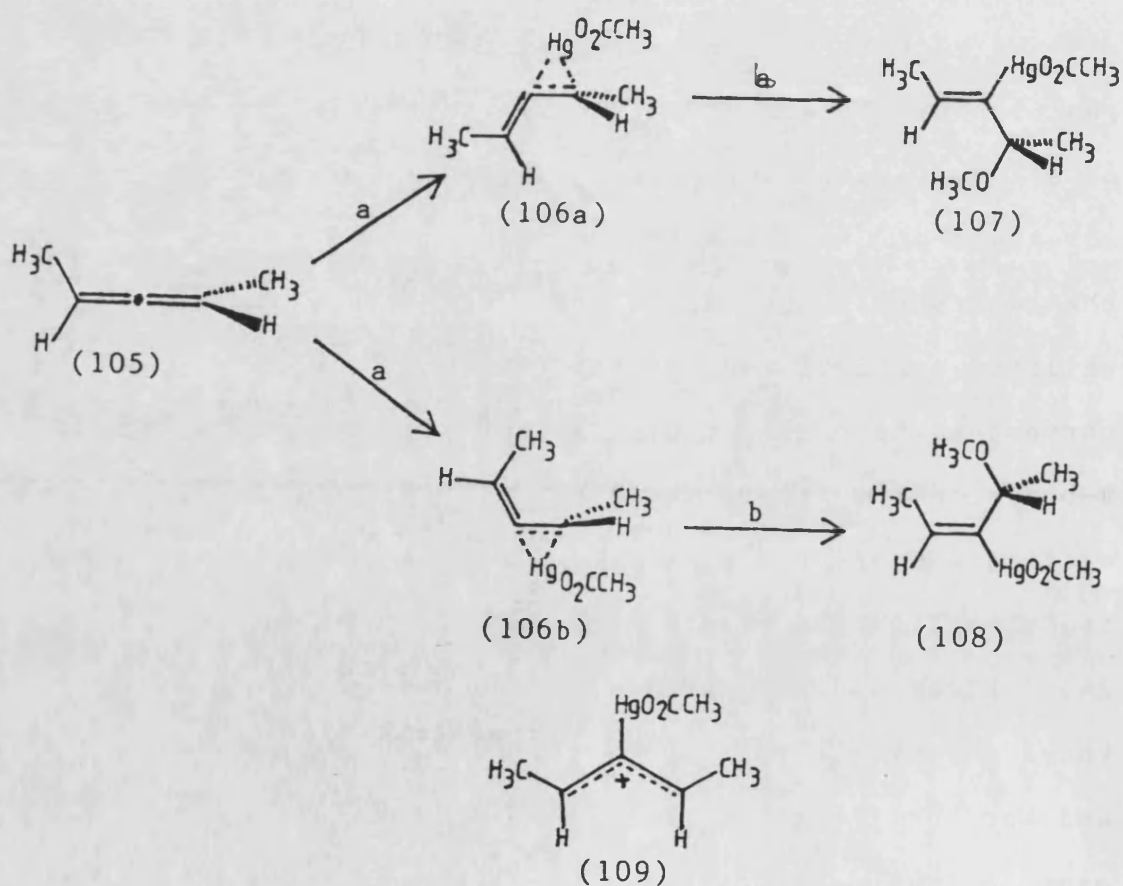
methods invoke either trans-metalation processes^{63,64} or homolysis of the organo-mercury bond to allow reductive coupling⁶⁵. The latter technique was applied by both Danishefsky⁶⁶ and Kozikowski⁶⁷ as a means of further functionalising the products of mercury(II) induced amine cycloadditions. Danishefsky succeeded in producing the disubstituted pyrrolidiny1 mercurial (97) by intramolecular aminomercuration, compounds such as (97) underwent reductive coupling with suitable alkenes to give addition products, for example (99) (scheme 23).

We were interested in further extending the general reaction of the electrophilically initiated cyclisation of alkenyl amines to allenic systems. The main advantage of incorporating an allenic function is that the heterocyclic product offers a greater synthetic utility when compared to that obtained from the corresponding olefinic cyclisation. In principle both π -bonds of the allene could be utilised, one to effect cyclisation and the other to allow for the further functionalisation of the heterocyclic product (scheme 24). Electrophilic addition reactions to allenes are known and have been studied for several electrophiles and variously substituted allenes⁶⁸. Consider, for example, the oxymercuration of allenes. Substituted allenes generally undergo addition of HgX at the central carbon whereas allene itself usually undergoes terminal



a) $\text{Hg}(\text{O}_2\text{CCH}_3)_2$; b) CH_3OH .

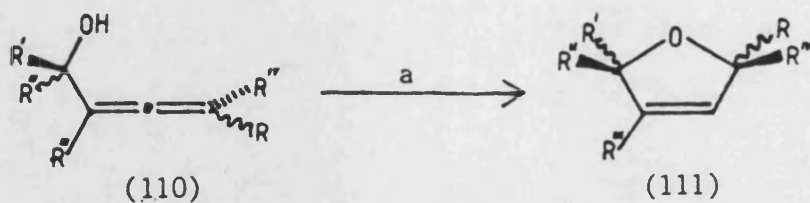
SCHEME 25



a) $\text{Hg}(\text{O}_2\text{CCH}_3)_2$; b) CH_3OH

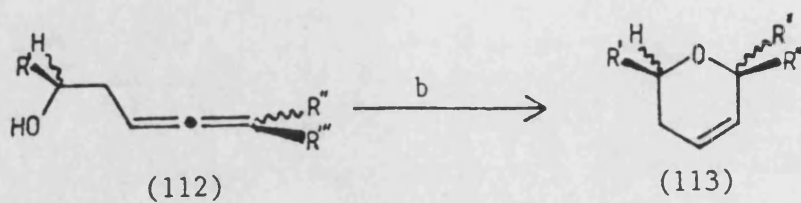
SCHEME 26

attack⁶⁷. The oxymercuration of 3-methylbuta-1,2-diene (100) with methanolic $\text{Hg}(\text{O}_2\text{CCH}_3)_2$ gives solely (102) rather than the more expected adduct (104)⁶⁸. This provides good evidence for the formation of a bridged mercurinium ion as an intermediate. Bonding between mercury and the terminal carbon must be strong enough in (101) to prevent rotation to the allylic intermediate (103). Further evidence for the preference of such ions as intermediates was gained by the methoxymercuration of R-(-)-penta-2,3-diene (105) which was formulated as shown in (scheme 26)⁷⁰. The vinylic mercurinium ions (106) could gain greater stability by opening and rotating to the allylic ion (109). The intermediate (109) would be expected to lead to racemic adducts from an optically active allene but (105) gave optically active (107) and (108). It should also be noted that the configuration of the asymmetric carbon in (107) showed that attack of methanol on (106a) was anti. However, the degree of stereospecificity observed in oxymercuration varied with the mercury salt employed⁷¹. In general it was observed that the higher the electronegativity of X in the electrophile HgX^+ the lower the stereospecificity. The highest level of activity retained in the product was obtained on addition of $\text{Hg}(\text{C}_2\text{H}_5)\text{Cl}$ and the lowest with HgCl_2 .



(110)	R'	R''	R'''	R''''	R	Yield%
a	H	H	H	CH ₃	<i>t</i> -C ₄ H ₉	63
b	H	H	CH ₃	H	<i>i</i> -C ₃ H ₇	55
c	H	<i>i</i> -C ₃ H ₇	H	CH ₃	CH ₃	61

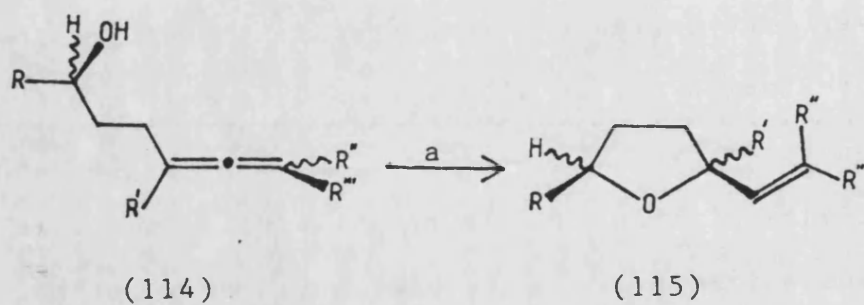
a) AgBF₄ (0.03 \equiv), CHCl₃, 20°C, 48h.



(112)	R'	R''	R'''	Yield%
a	H	CH ₃	CH ₃	65
b	CH ₃	CH ₃	C ₂ H ₅	63
c	H	<i>n</i> -C ₃ H ₇	H	69

b) AgNO₃ (0.1 \equiv), H₂O, Dioxane, CaCO₃, 50h.

SCHEME 27



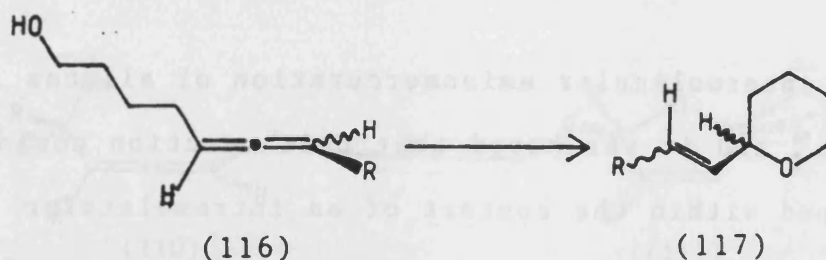
(114)	R	R'	R''	R'''	Yield%
a	H	H	CH ₃	CH ₃	95
b	CH ₃	CH ₃	H	H	95
c	H	CH ₃	H	H	75

a) AgNO₃ (1.2 \equiv), H₂O, 20°C, 5h.

SCHEME 28

The intermolecular aminomercuration of allenes is also known⁶⁸, and it was hoped that this reaction could be developed within the context of an intramolecular addition. There has already been a certain amount of success in effecting the cyclisation of a variety of allenic alcohols and amines, especially when silver(I) salts were employed as electrophiles. Claesson and Olsson reported the electrophilically initiated cyclisation of α -allenic alcohols (110) and β -allenic alcohols (112) to give dihydrofurans (111) and 5,6-dihydro-2H-pyrans (113) respectively (scheme 27)⁷². Gore reported the synthesis of tetrahydrofurans (115) by the cyclisation of γ -allenic alcohols (114) with silver nitrate (scheme 28)⁷³. Finally δ -allenic alcohols were cyclised by Gore (scheme 29)⁷⁴ and Gallagher (scheme 30)⁷⁵ to give tetrahydropyrans. Gallagher found that α -substituted δ -allenic alcohols (118) cyclised to give predominantly the cis-product (121) and this presumably reflects a preference for a chair like transition state (120). δ -Allenic amines have been cyclised to give piperidines (scheme 31)⁷⁶, and γ -allenic amines yield pyrrolidines (scheme 32)⁷⁷.

In terms of our proposed synthesis of anatoxin-a, (scheme 15), we predicted that the 2,5-disubstituted-pyrrolidine (126) could be prepared by the functionalisation of the proline derivative (127). It seemed

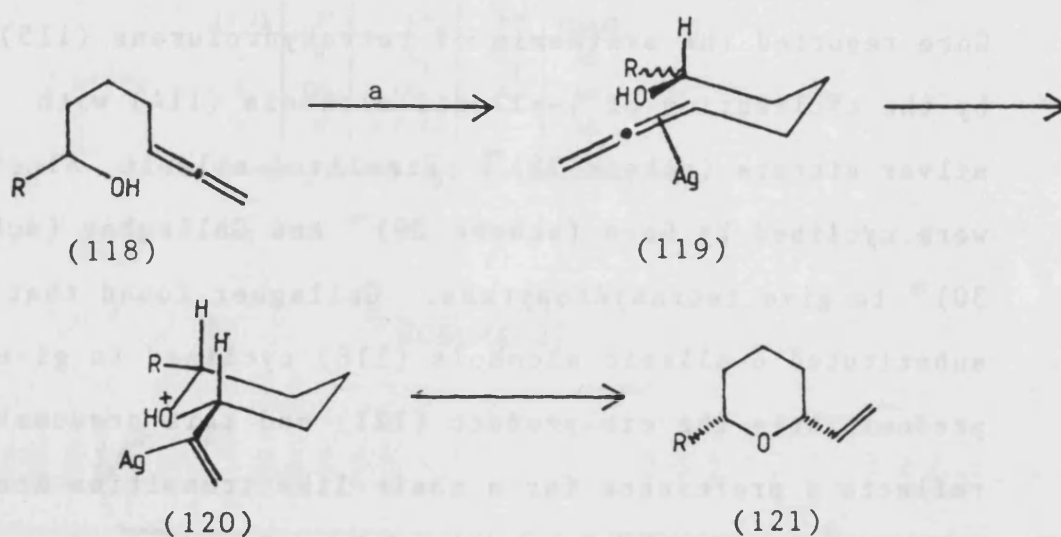


(116)	R	Conditions	Yield%
a	H	a	95
b	C ₂ H ₅	b	90

a) AgNO₃ (2%), H₂O, Acetone, 20°C, 24h

b) AgNO₃ (2%), H₂O, Acetone, 60°C, 7h

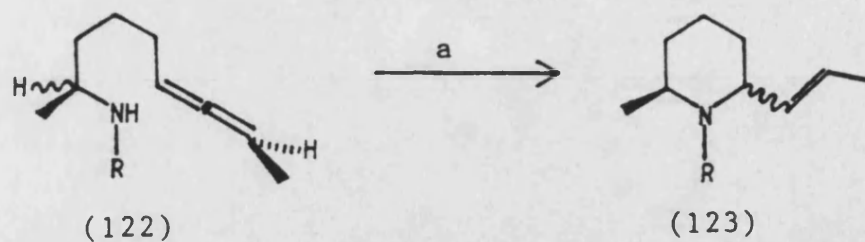
SCHEME 29



(118)	R	Cis/Trans	Yield%
a	CH ₃	18	75
b	t-C ₄ H ₉	≥25	50
c	C-C ₆ H ₁₁	13	97
d	C ₆ H ₅	30	93
e	CH:CH ₂	≥25	50

a) AgNO₃ (1.2%), H₂O, Acetone, 50°C, 4-8h.

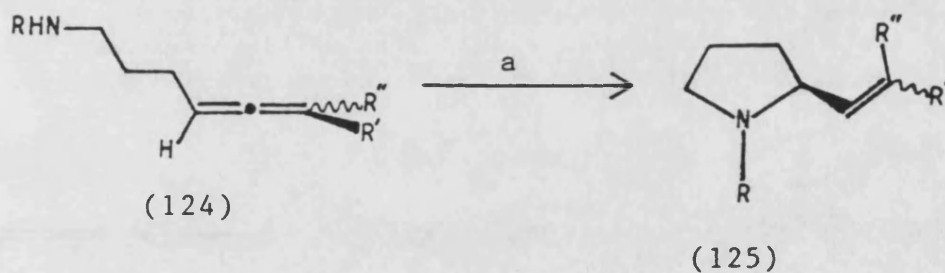
SCHEME 30



(122)	R	Cis/Trans	Yield%
a	CH ₂ C ₆ H ₅	1	100
b	H	1.6	100

a) AgNO₃ (0.3%), H₂O, Acetone, 25°C.

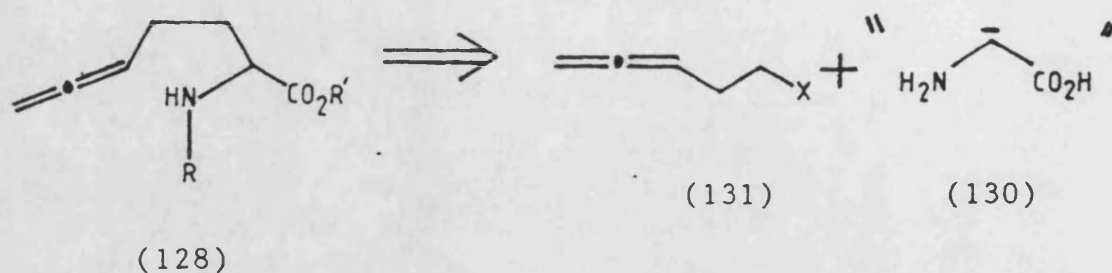
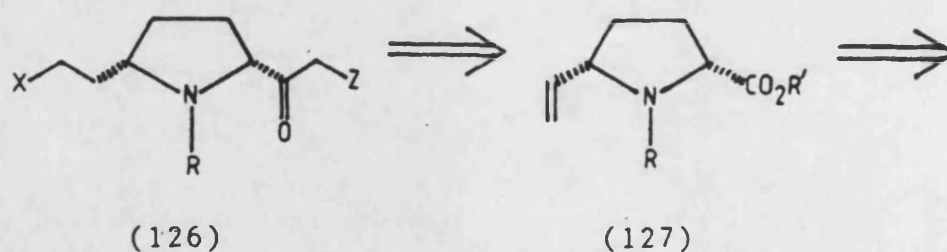
SCHEME 31



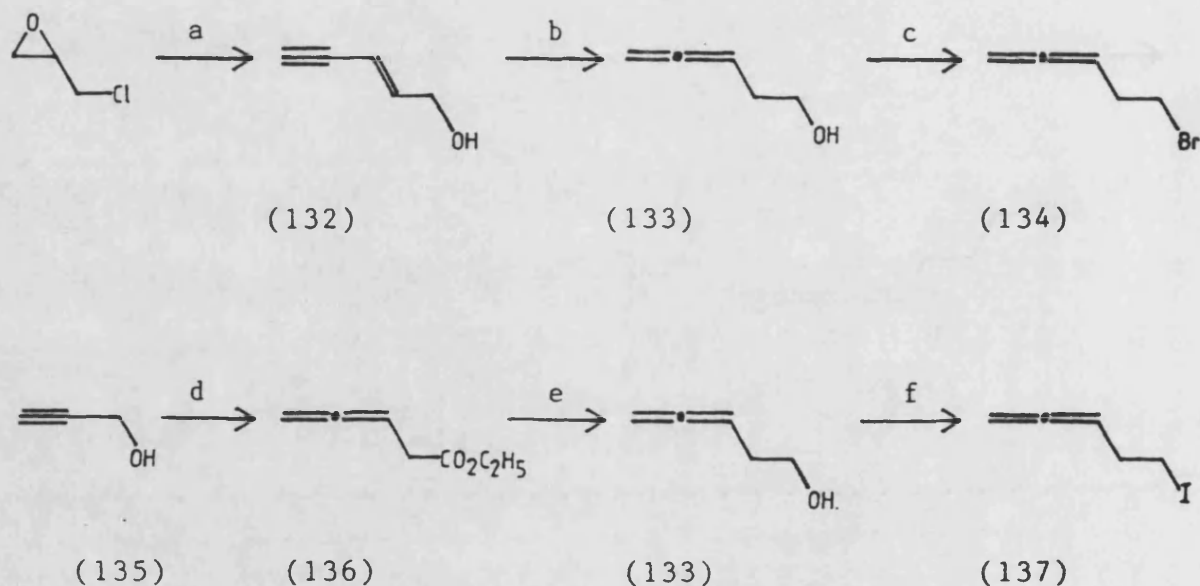
(124)	R	R'	R''	Yield
a	CH ₂ C ₆ H ₅	H	H	79
b	CH ₂ C ₆ H ₅	CH ₃	CH ₃	79

a) AgNO₃ (2%), H₂O, Acetone.

SCHEME 32



SCHEME 33



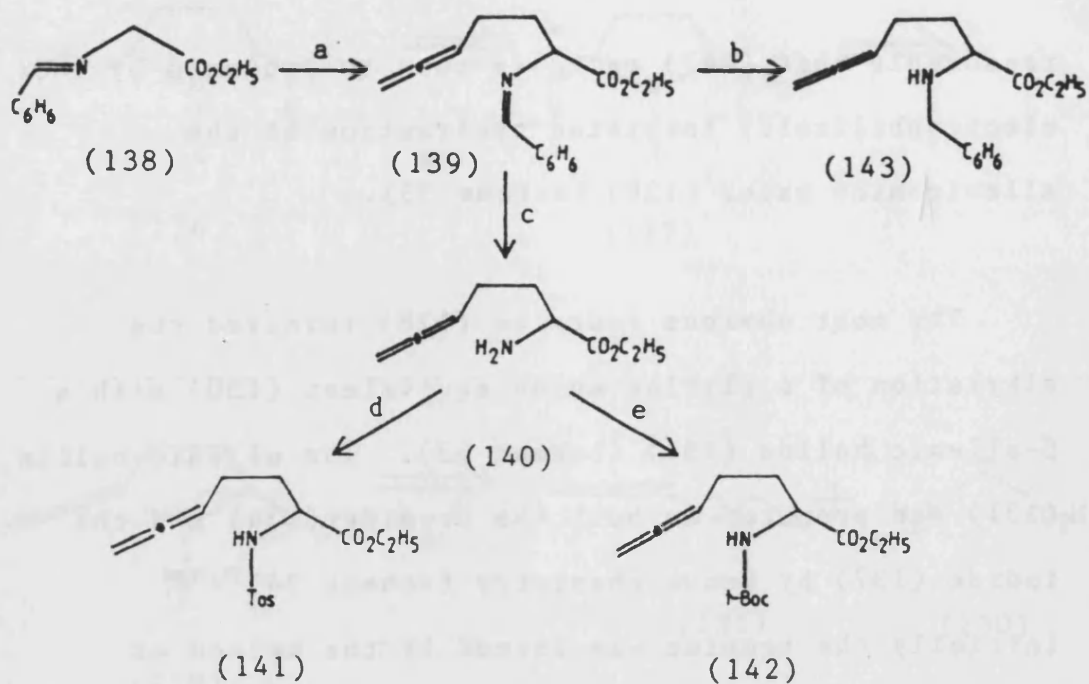
a) $\text{NaC}\equiv\text{CH}$, NH_3 , 42%; b) LiAlH_4 , $(\text{C}_2\text{H}_5)_2\text{O}$, 83%; c) $(\text{PhO})_3\text{PBr}_2$, pyridine, 59%; d) $(\text{C}_2\text{H}_5\text{O})_3\text{CCH}_3$, H^+ , 11%;
 e) LiAlH_4 , $(\text{C}_2\text{H}_5)_2\text{O}$, -78°C , 74%; f) (i) $\text{CH}_3\text{SO}_2\text{Cl}$, CH_2Cl_2 , $(\text{C}_2\text{H}_5)_3\text{N}$; (ii) NaI , acetone, 56°C , 69%.

SCHEME 34

reasonable that (127) could in turn be produced by the electrophilically initiated cyclisation of the allenic amino ester (128). (scheme 33).

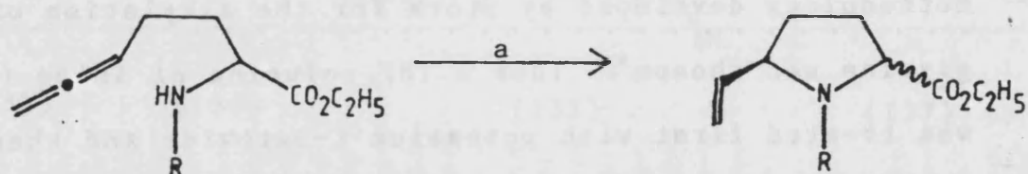
The most obvious route to (128) involved the alkylation of a glycine anion equivalent (130) with a β -allenic halide (131) (scheme 33). The allenic halide (131) was prepared as both the bromide (134) and the iodide (137) by known chemistry (scheme 34)^{78,79,80}. Initially the bromide was formed by the method of Landor incorporating the conversion of alcohol (133) to bromide (134) by treatment with triphenylphosphite dibromide⁷⁹. However this reaction proved capricious and although yields of up to 59% were recorded, they were often much lower. As a result we employed Dauben's route to the allenic iodide (137) as this proved to be more reliable⁸⁰.

To prepare the allenic amino ester (128) the methodology developed by Stork for the alkylation of glycine was chosen⁸¹. Thus a THF solution of imine (138) was treated first with potassium t-butoxide and then either allenic bromide (134) or allenic iodide (137). This gave (139) which was isolated, then immediately hydrolysed with 2M hydrochloric acid to give primary amine (140). The amine (140) proved to be unstable but could be protected as either the p-toluenesulphonamide (141) or the t-butylcarbamate (142) derivative, both



a) (i) $(\text{CH}_3)_3\text{COK}$, THF, -78°C , (ii) 5-iodopenta-1,2-diene (137); b) NaBH_4 , CH_3OH , 60%; c) aq HCl d) $p\text{-CH}_3\text{C}_6\text{H}_4\text{SO}_2\text{Cl}$, pyridine, 66%, e) $\text{O}(\text{CO}_2\text{C}(\text{CH}_3)_3)_2$, CH_2Cl_2 , 49%

SCHEME 35



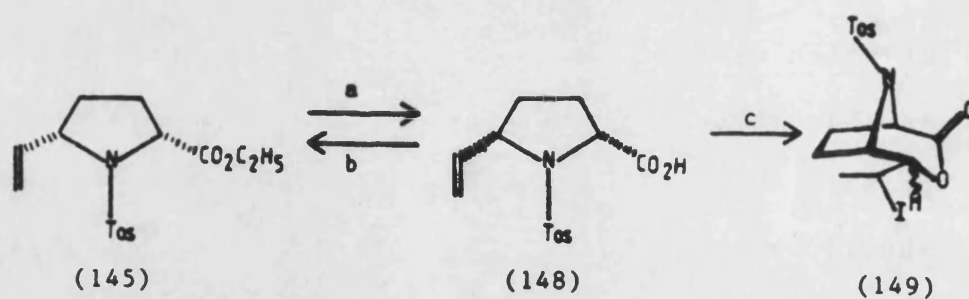
Adduct	R	Product	Cis/Trans	Yield%
140	H	144	1	60
141	$p\text{-CH}_3\text{C}_6\text{H}_4\text{SO}_2$	145	≥ 50	98
142	$(\text{CH}_3)_3\text{CO}_2\text{C}$	146	≥ 50	88
143	$\text{C}_6\text{H}_5\text{CH}_2$	147	≥ 50	93

a) AgBF_4 (0.1-1.0 \equiv)

SCHEME 36

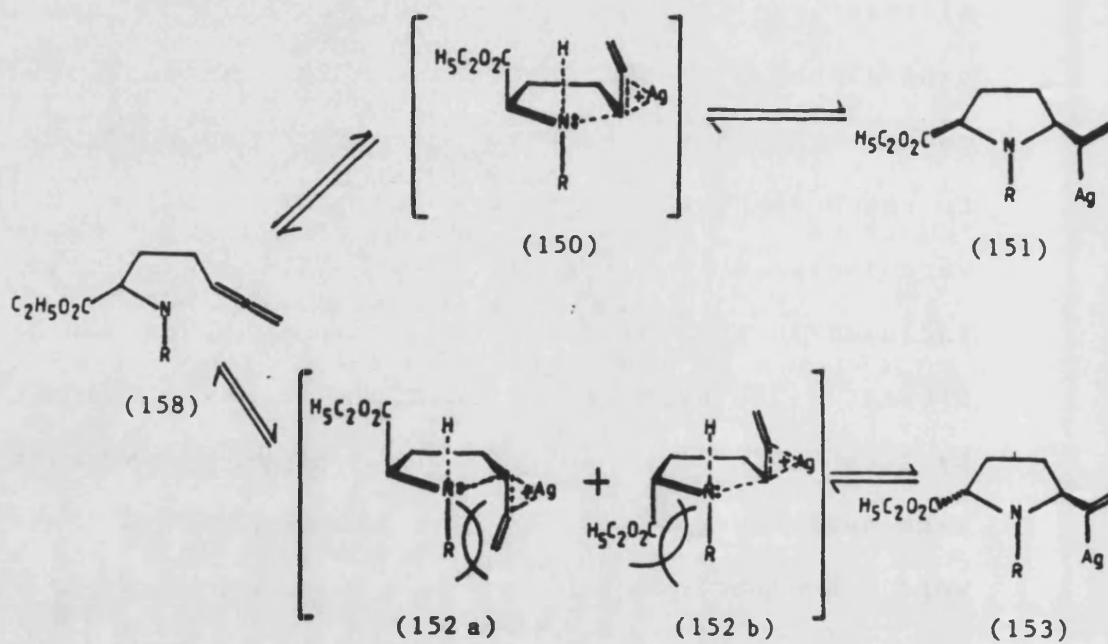
of which were stable crystalline solids (scheme 35)⁸². Another member of the group at Bath isolated the primary amine (140) and the secondary amine (143)⁸³. It should be noted that in all cases the alkylated glycine derivatives produced by this method were racemic.

With the allenes (141) and (142) in hand the silver(I) initiated cyclisation of these derivatives were examined. Methylene chloride solutions of the allenes were treated with silver tetrafluoroborate (1 equivalent), at room temperature, under anhydrous conditions and in the dark. Reaction was generally seen to reach completion within a few hours and the products were isolated in a standard way; a brine wash was included in the work up to remove the silver salts present. The heterocyclic product was purified either by flash chromatography or, when larger scale reactions were performed, simply by crystallization. The reactions were also carried out using 0.1 equivalents of silver tetrafluoroborate and worked up in a similar fashion, though complete conversion of the allenes to their corresponding cyclic products took several days. In both the catalytic and stoichiometric cases quantitative yields of the cis-2,5-disubstituted pyrrolidines (145) and (146) were observed. Another worker at Bath cyclised the amino allenes (140) and (143) to give the disubstituted pyrrolidines (144) and (147)⁹¹. It was interesting to note that although the secondary amine (143)



a) LiOH, H₂O, THF, 72%; b) C₂H₅COCl, C₂H₅OH, 0°C, 79%;
c) I₂, KI, NaHCO₃, H₂O;

SCHEME 37

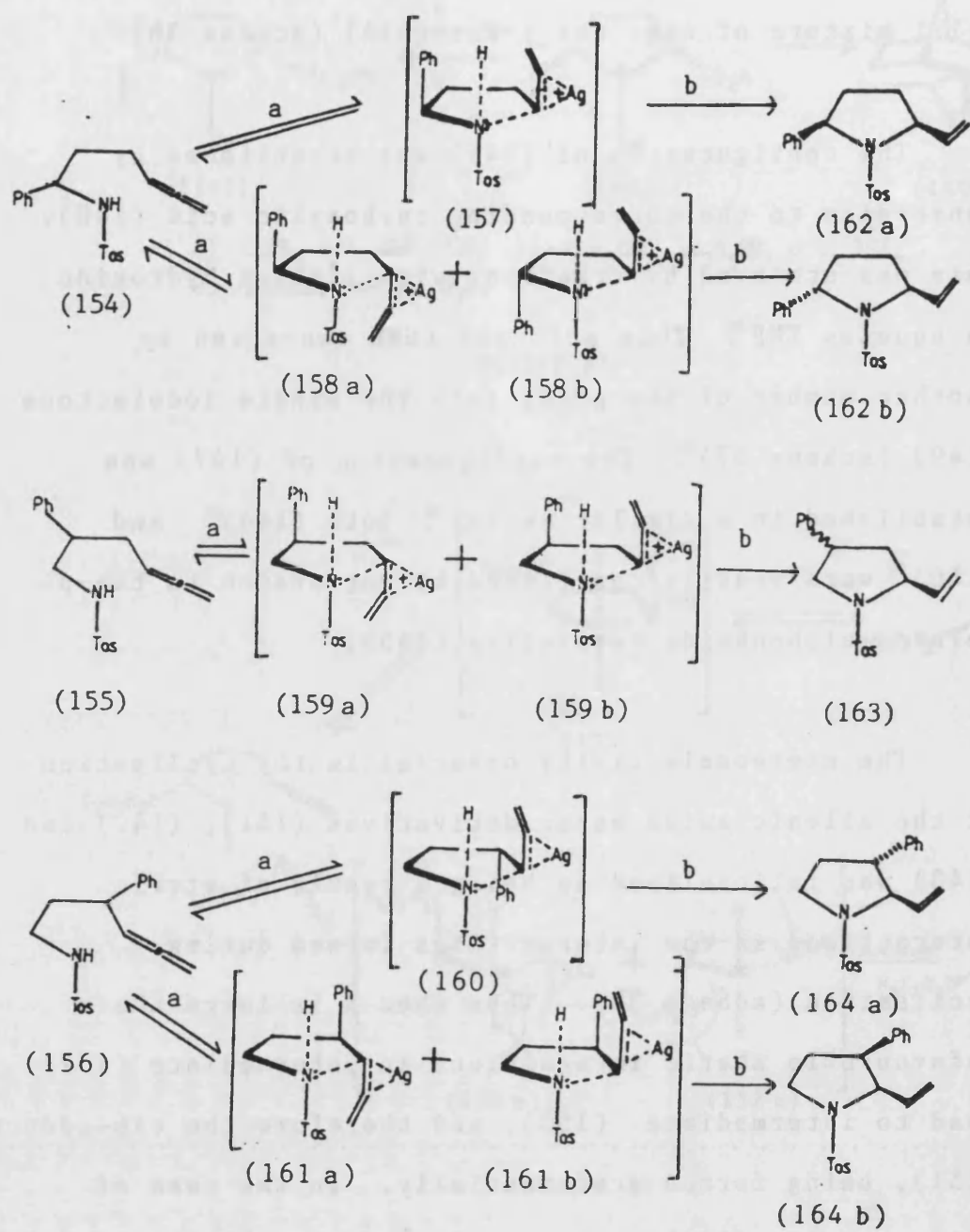


SCHEME 38

gave purely the cis-adduct (147) the primary amine gave a 1:1 mixture of cis- and trans-(144) (scheme 36).

The configuration of (145) was established by conversion to the corresponding carboxylic acid (148). This was achieved by treatment with lithium hydroxide in aqueous THF⁸². This acid was then converted by another member of the group into the single iodolactone (149) (scheme 37)⁸³. The configuration of (147) was established in a similar manner⁸³. Both (144)⁸³ and (146)⁸⁴ were readily correlated by conversion to the p-toluenesulphonamide derivative (145).

The stereoselectivity observed in the cyclisation of the allenic amino ester derivatives (141), (142) and (143) was rationalised as being a result of steric interactions in the intermediates formed during cyclisation (scheme 38). Thus when R is large the unfavourable steric interactions in intermediate (152) lead to intermediate (150), and therefore the cis-adduct (151), being formed preferentially. In the case of primary amine (140) there are no unfavourable steric interactions in either intermediate (150) or (152). As a result neither intermediate is formed preferentially and the reaction is nonstereoselective.

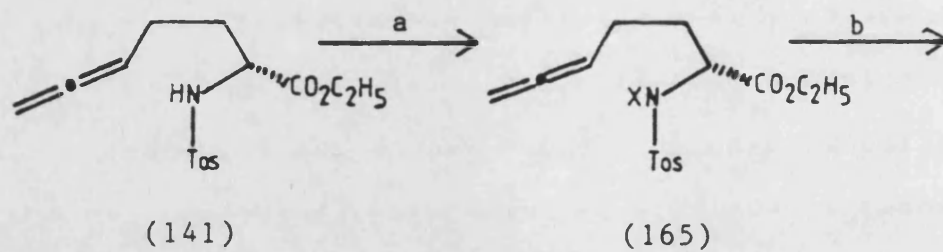


a) AgBF_4 ; b) H_2O .

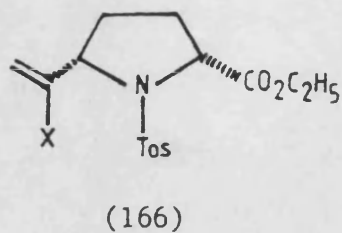
SCHEME 39

In an attempt to provide further evidence supporting this rationale, additional work is currently under investigation within the group. Due to the relevance of this work, it shall be discussed here briefly. The objective was to prepare the allenic sulphonamides (154), (155) and (156) and, by treatment with silver tetrafluoroborate, effect cyclisation to give the pyrrolidines (162), (163) and (164) (scheme 39). The allenic sulphonamide (154) cyclised to give predominantly the cis-disubstituted pyrrolidine (162a)⁸⁵. This would be expected due to the unfavourable steric interactions present in intermediate (158). In the case of (155) no stereoselectivity is observed upon silver(I) catalysed cyclisation⁸⁶. This is presumably a result of the phenyl substituent being sufficiently removed from other substituents in intermediates (159), so that steric interactions are negligible. It still remains to prepare and cyclise (156)⁸⁷. However, for the rationale to be upheld, then unfavourable steric interactions in intermediate (161) should lead to the formation of the trans-adduct (164a) predominantly.

To further justify the intermediates proposed, the author attempted to follow the cyclisation of sulphonamide (141) by ¹H nmr. To maximise the concentration of any intermediate present, a stoichiometric amount of silver tetrafluoroborate was required. However, the silver salt was insufficiently soluble in methylene



X = 64% D, 36% H.



X = 62% D, 38% H.

a) D₂O, b) AgBF₄, 95%.

(166) δ_{H} (270 MHz, CDCl₃)

$\leftarrow \delta$ 5.38 (dt, J 17.0, 1.3 Hz)

$\leftarrow \delta$ 5.09 (dt, J 10.1, 1.3 Hz)

$\leftarrow \delta$ 5.37 (broad s)

$\leftarrow \delta$ 5.09 (broad s)

SCHEME 40

chloride or chloroform for this to be possible. Silver tetrafluoroborate is soluble in acetonitrile but no reaction was observed when (141) was treated with the silver salt in this solvent, even when heated.

Acetonitrile is well known to strongly chelate silver(1)⁸⁸, therefore in this solvent the electrophile is presumably unavailable to the allenic substrate.

To help ascertain the fate of the carbon-silver bond in the putative intermediate (151), we attempted the cyclisation of allenic sulphonamide (141) in a deuterated solvent. The reaction was conducted in CDCl_3 with 0.5 equivalents of silver tetrafluoroborate. The cis-disubstituted proline (145) was formed exclusively with no incorporation of deuterium observed in the product. This being the case, we deduced that homolysis of the carbon-silver bond did not occur during the course of the reaction.

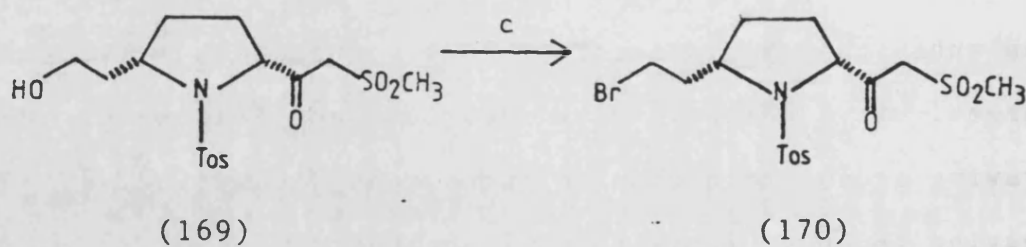
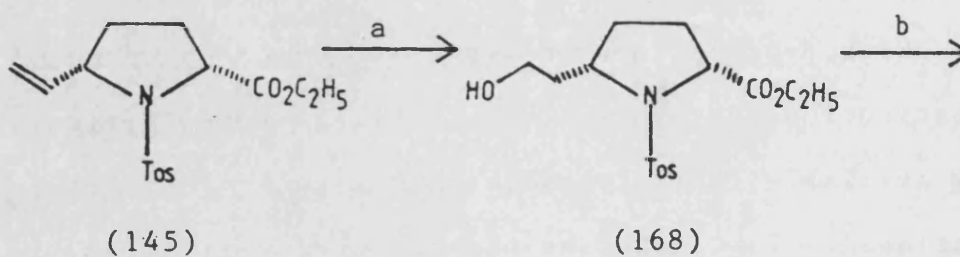
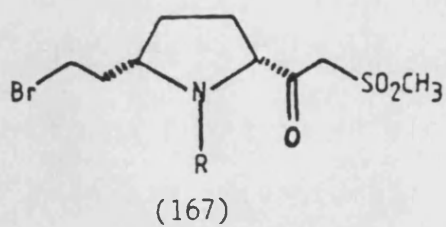
In a further effort to increase our understanding of the fate of this silver-carbon bond, the proton bound to nitrogen in the allenic substrate (141) was exchanged for deuterium prior to cyclisation (scheme 40). ^1H nmr showed complete deuteration had not occurred. However, partial disappearance of the NH signal, at δ 5.25, indicated that 64% of the sulphonamide (165) had been deuterated on nitrogen. Treatment of this material

with a stoichiometric amount of silver tetrafluoroborate gave the cis-disubstituted proline (166) in 95% yield.

^1H nmr indicated that 62% of the adduct was deuterated at C1 of the ethenyl substituent. The full implication of this experiment will become apparent when more is known about the mechanism of the cyclisation. Although the protonation of the proposed intermediate is not necessarily an intramolecular process, this possibility can not be excluded.

With pyrrolidines possessing the correct substitution pattern and the required stereochemistry now available, the proposed synthesis of anatoxin-a was continued. To synthesise the required 9-azabicyclo[4.2.1]-nonane system, further functionalisation of the substituents on the pyrrolidine ring was necessary (scheme 15). The substituted pyrrolidine (167) was chosen as a suitable target. This molecule contained both an electron withdrawing group, to aid anion formation, and a good leaving group to facilitate ring closure.

We initially attempted to synthesise the p-toluene-sulphonamide derivative of (167) by a series of reactions (scheme 41). The first stage of which involved the anti-Markovnikov addition of water across the double bond in olefin (145). This was achieved by treating the olefin with diborane, followed by oxidation of the resulting



a) (i) $\text{BH}_3 \cdot \text{THF}$; (ii) H_2O_2 , NaOH , 79%; b) $\text{LiCH}_2\text{SO}_2\text{CH}_3$, THF , 63%;
 c) $(\text{C}_6\text{H}_5)_3\text{PBr}_2$, THF , 78%.

SCHEME 41

alkyl borane with alkaline hydrogen peroxide⁸⁹. Diborane is commercially available as a complex with tetrahydrofuran (THF-BH₃) or as a complex with dimethyl sulphide ((CH₃)₂S-BH₃). When commercial THF-BH₃ was employed as the hydroborating agent, the alcohol (168) was synthesised in yields of up to 80%. Upon storage the commercial reagent proved unreliable, with yields for the reaction falling significantly. The commercial (CH₃)₂S-BH₃ complex gave only a 23% yield of (168) when it was employed as the hydroborating agent. This is possibly due to the greater stability of the (CH₃)₂S-BH₃ complex necessitating more vigorous conditions. The most reliable results were obtained when the THF-BH₃ complex was generated freshly with yields of 79% being obtained consistently.

The hydroxy ester (168) was further functionalised to the β -keto sulphone (169) (scheme 41). This was accomplished by treating the ester with the conjugate base of dimethyl sulphone⁹⁰. The required carbanion was formed by the addition of n-butyllithium to a THF solution of dimethyl sulphone. Care had to be taken that the sulphone substrate was fully dissolved before addition of the base, otherwise complete deprotonation did not occur. In this event the presence of n-butyllithium in the reaction mixture resulted in epimerisation of the adduct. However, when care was taken over the formation of the carbanion, the keto sulphone (169) was

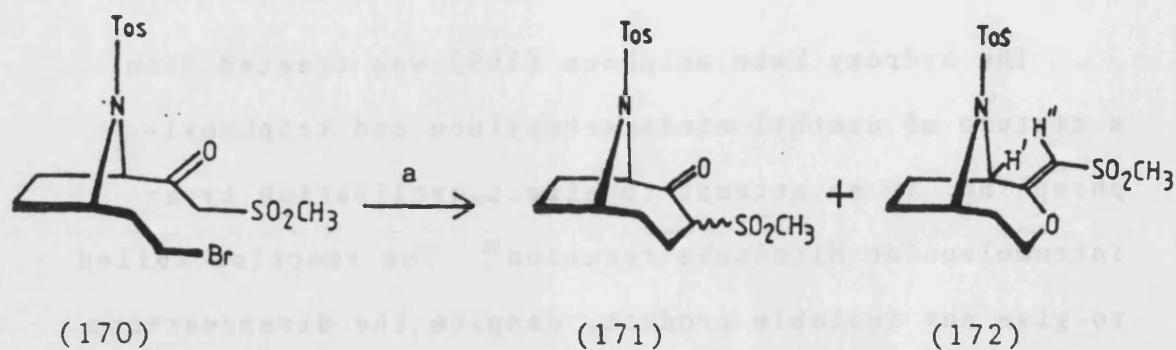
formed in 63% yield.

The hydroxy keto sulphone (169) was treated with a mixture of diethyl azodicarboxylate and triphenylphosphine in an attempt to effect cyclisation by an intramolecular Mitsunobu reaction⁹¹. The reaction failed to give any isolable product, despite the disappearance of the starting material being observed by tlc.

In an attempt to activate the hydroxy function of (169) to nucleophilic displacement, we decided to form the bromide (170). This was effected in 78% yield by treatment of (169) with triphenylphosphine dibromide⁹².

A similar series of reactions was attempted on the t-butylcarbamate derivative (146). Although initial studies suggested that the reactions did proceed, yields were significantly lower and the products more difficult to handle than in the corresponding sulphonamide cases. As a result, the t-butylcarbamate series of reactions was abandoned and only the sulphonamide series pursued further.

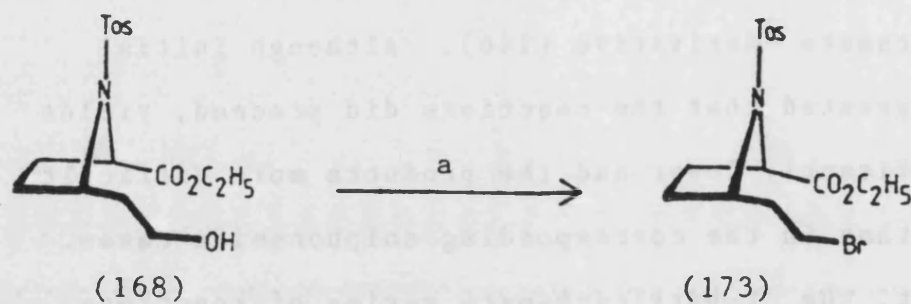
With the production of the target compound (170) we then desired to effect cyclisation to produce the 9-azabicyclo[4.2.1]nonane ring skeleton. When bromide (170) was treated with either lithium diisopropylamide



a) NaH, $(\text{CH}_3)_2\text{SO}$, 83%

9% nOe enhancement
observed between H'
and H''

SCHEME 42

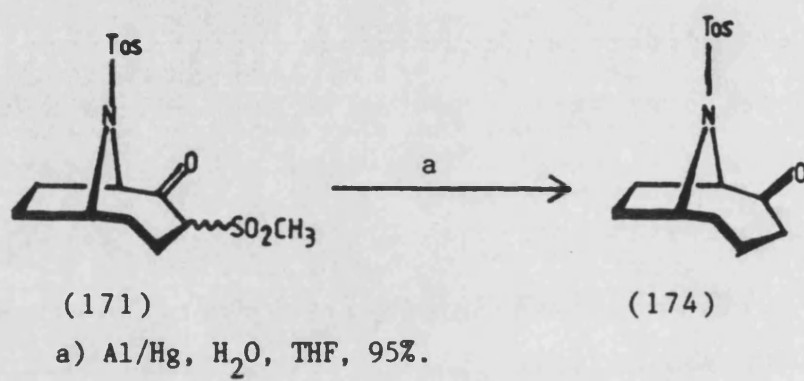


a) $(\text{C}_6\text{H}_5)_3\text{PBr}_2$, THF 90%

SCHEME 43

or potassium hexamethyldisilazide cyclisation was not observed. If (170) was added to a solution of dimsyl sodium then the bromide was effectively destroyed, again with no sign of cyclisation. However, if solid sodium hydride was added to a dimethyl sulphoxide solution of (170), heated to 30°C, then complete cyclisation was seen to occur (scheme 42). The bicyclic keto sulphone (171) was formed as a colourless crystalline solid in 83% yield, the structure of which was confirmed by X-ray structural analysis (see appendix 1). The reaction also gave a 4% yield of the O-alkylated product (172). This was shown to be purely the Z isomer by nOe enhancement.

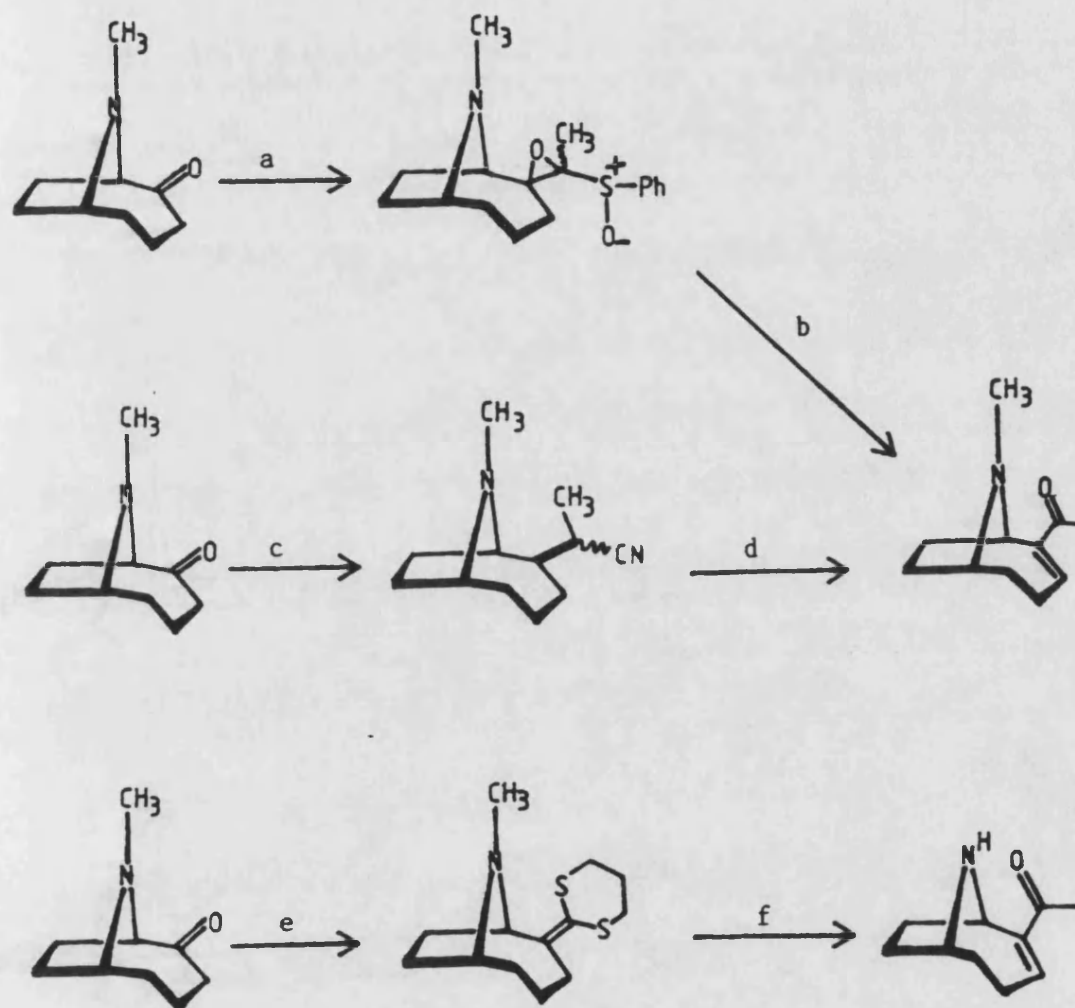
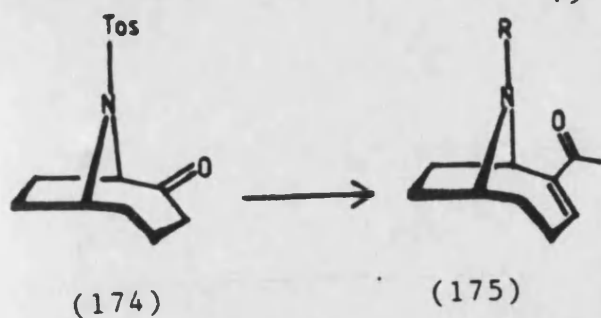
In an unsuccessful attempt to synthesise the bicyclic keto sulphone (171) more directly, the bromide (173) was prepared. This was attained in 90% yield by the addition of the hydroxy ester (168) to triphenylphosphine dibromide (scheme 43)⁹². A THF solution of (173) was treated with two molar equivalents of the anion of dimethyl sulphone. As a result, the keto sulphone (170) was formed with no sign of cyclisation observed. In an alternative attempt to initiate cyclisation, (173) was added to one molar equivalent of the anion of dimethyl sulphone. This mixture was then heated to 40°C and treated with one molar equivalent of dimsyl sodium. The starting material was destroyed and no recognisable products were isolated.



SCHEME 44

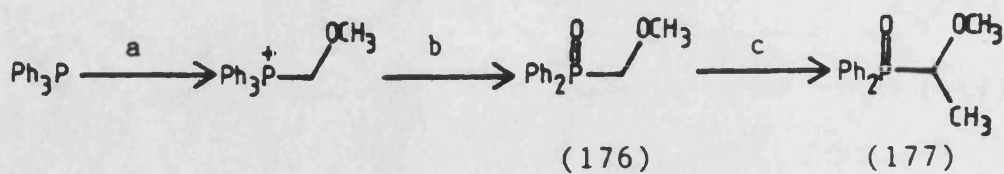
The keto sulphone (171) was readily reduced to the bicyclic ketone (174) by treatment with aluminium amalgam (scheme 44)⁹³. With the synthesis of this ketone complete, it only remained to form the homologous α,β -unsaturated methyl ketone (175) (scheme 45). Other workers have already achieved this homologation for cases where the ketone (173) bears different substituents on nitrogen^{31,36,94}. These efforts have been illustrated in (scheme 45).

We decided to develop a different technique for forming the required α,β -unsaturated ketone. This would further expand the synthetic possibilities available for producing analogues of anatoxin-a. As such, we do not intend to suggest that this route is the best way of completing the synthesis, but merely another alternative. The method adopted was an adaptation of the Wittig reaction. The phosphine oxide (176), prepared as described by Warren⁹⁵, was alkylated by deprotonation with lithium diisopropylamide followed by addition of methyl iodide. Careful crystallisation of the adduct from toluene and a small amount of light petroleum gave (177) as a colourless crystalline solid (scheme 46). We hoped to separately treat both phosphine oxides (176) and (177) with ketone (174), under Horner Emmons conditions⁹⁶, to give the enol ethers (179) and (181) respectively (scheme 47). This was first achieved for



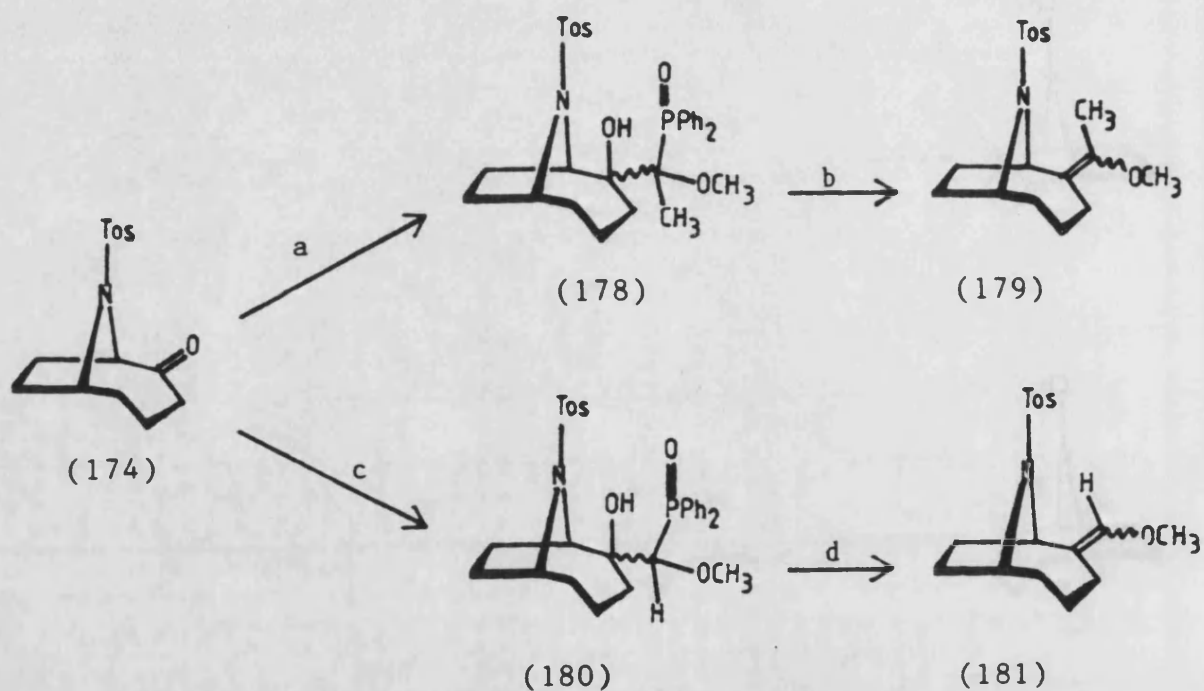
a) (i) $n\text{-BuLi}$, $\text{CH}_3\text{ClCHS(O)C}_6\text{H}_5$, THF, 90%; (ii) $t\text{-BuOK}$, THF, 90%; b) toluene, reflux, 35%;^a c) NaNH_2 , $(\text{C}_2\text{H}_5\text{O})_2\text{P(O)CH(CH}_3\text{)CN}$, THF, 64%; d) (i) lithium diisopropylamide, HMPA, THF, O_2 ; (ii) 1M aq Na_2SO_3 , 43%;^a e) $n\text{-BuLi}$, 2-(trimethylsilyl)-1,3-dithiane, THF, 78%; f) (i) $n\text{-BuLi}$, HMPA, CH_3I , -78°C , 85%; (ii) $\text{ClCO}_2\text{CH:CH}_2$, CH_2Cl_2 , reflux, 83%; (iii) 3.6M aq HCl , DMSO, dioxane, reflux; (iv) NaOH , $(\text{C}_2\text{H}_5)_2\text{O}$, 64%;^a

SCHEME 45



a) $\text{ClCH}_2\text{OCH}_3$, C_6H_6 , 50°C , 96 h; b) NaOH , H_2O , reflux, 90%
 c) Lithium diisopropylamide, CH_3I , 39%.

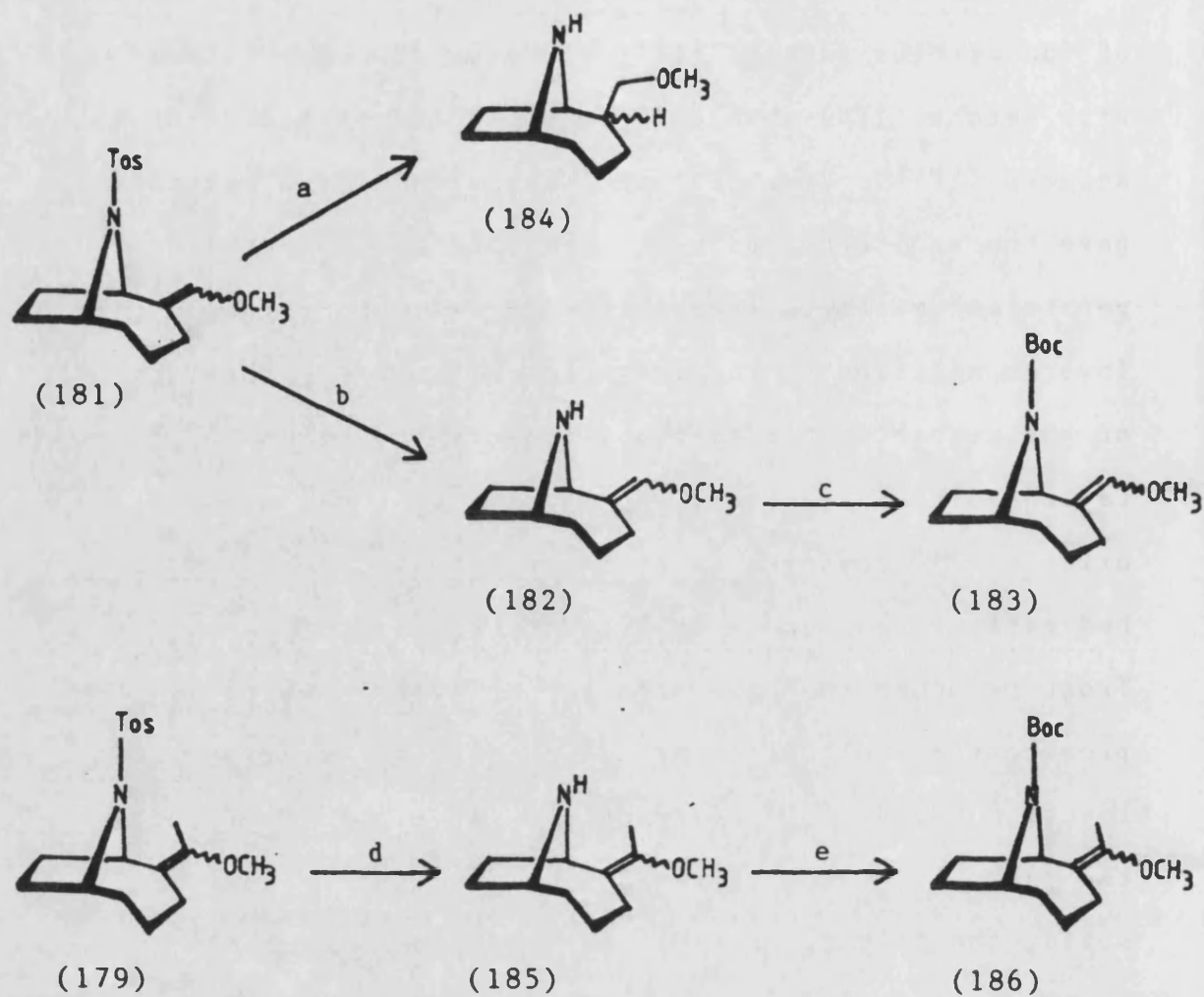
SCHEME 46



a) $\text{Li}^+((\text{C}_6\text{H}_5)_2\text{P}(\text{O})\text{C}(\text{CH}_3)\text{OCH}_3)^-$, DME, -78°C ; b) NaH , THF, 97%;
 c) $\text{Li}^+((\text{C}_6\text{H}_5)_2\text{P}(\text{O})\text{CHOCH}_3)^-$, DME, -78°C ; d) NaH , THF, 95%.

SCHEME 47

the methylated phosphine oxide (177). A THF solution of the lithium salt of (177) was treated consecutively with ketone (174) then HMPA to yield the diastereomeric adducts (178). Treatment of (178) with sodium hydride gave the enol ether (179) in 19% yield. However, persistent attempts to repeat this reaction failed. Inverse addition of the anion to the ketone or addition of the ketone to the cerium(III) salt of the anion⁹⁷ also failed to give reaction. The purity of the phosphine oxide (177), employed in Wittig reactions of this type had earlier been shown by Trost to be imperative⁹⁸. Trost proposed that the presence of impurities could promote the enolisation of ketone substrates, therefore lowering yields for the reaction. As we had prepared the phosphine oxide (177) as a colourless crystalline solid, the melting point of which agreed with that reported by Trost⁹⁸, we believed that this was not the problem in our case. Instead, difficulties in repeating the reaction were thought to be a result of the instability of the anion in THF. In an attempt to redress this, the reaction was repeated in DME and under an atmosphere of argon⁹⁹. The diastereomeric adducts (180) were then formed consistently and, after base treatment, gave the enol ether (179) in 97% yield. Occasionally incomplete alkylation was observed during the synthesis of the phosphine oxide (177). This led to the adduct being contaminated with the unalkylated phosphine oxide (176). If it was attempted to synthesise the enol ether (179)

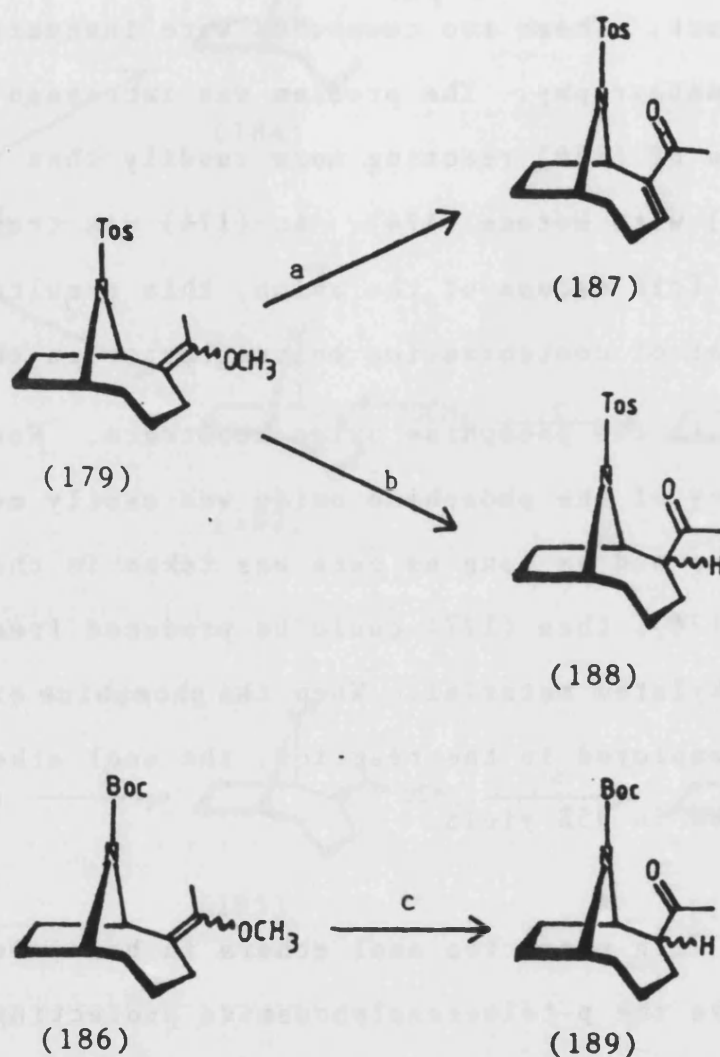


a) Li, NH₃, -33°C, 66%; b) Li, NH₃, -78°C, 85%; or ((CH₃OCH₂CH₂O)₂-AlH₂)Na, toluene, reflux, 58%; c) ((CH₃)₃CO₂C)₂O, CH₃OH, 100%; d) Li, NH₃, -78°C, 55%; e) ((CH₃)₃CO₂C)₂O, CH₃OH, 100%;

SCHEME 48

with this material, then although (179) was formed, traces of the enol ether (178) were present in the product. These two compounds were inseparable by flash chromatography. The problem was increased due to the anion of (176) reacting more readily than the anion of (177) with ketone (174). As (174) was treated with a four fold excess of the anion, this resulted in the extent of contamination being greater in the product than in the phosphine oxide substrate. However, the purity of the phosphine oxide was easily monitored by ^1H nmr and as long as care was taken in the alkylation of (176), then (177) could be produced free of the unalkylated material. When the phosphine oxide (176) was employed in the reaction, the enol ether (181) was formed in 95% yield.

With these two enol ethers in hand, we wanted to cleave the p-toluenesulphonamide protecting group, present in both, to give the secondary amines (182) and (185) (scheme 48). The deprotection of (181) was achieved by treatment of the sulphonamide with either sodium bis(2-methoxyethoxy)aluminium hydride in refluxing toluene¹⁰⁰ or lithium in liquid ammonia at -78°C . The temperature of the latter reduction was found to be crucial. If the reaction was conducted at -33°C , then reduction of the carbon-carbon double bond as well as sulphonamide cleavage occurred, to give amine

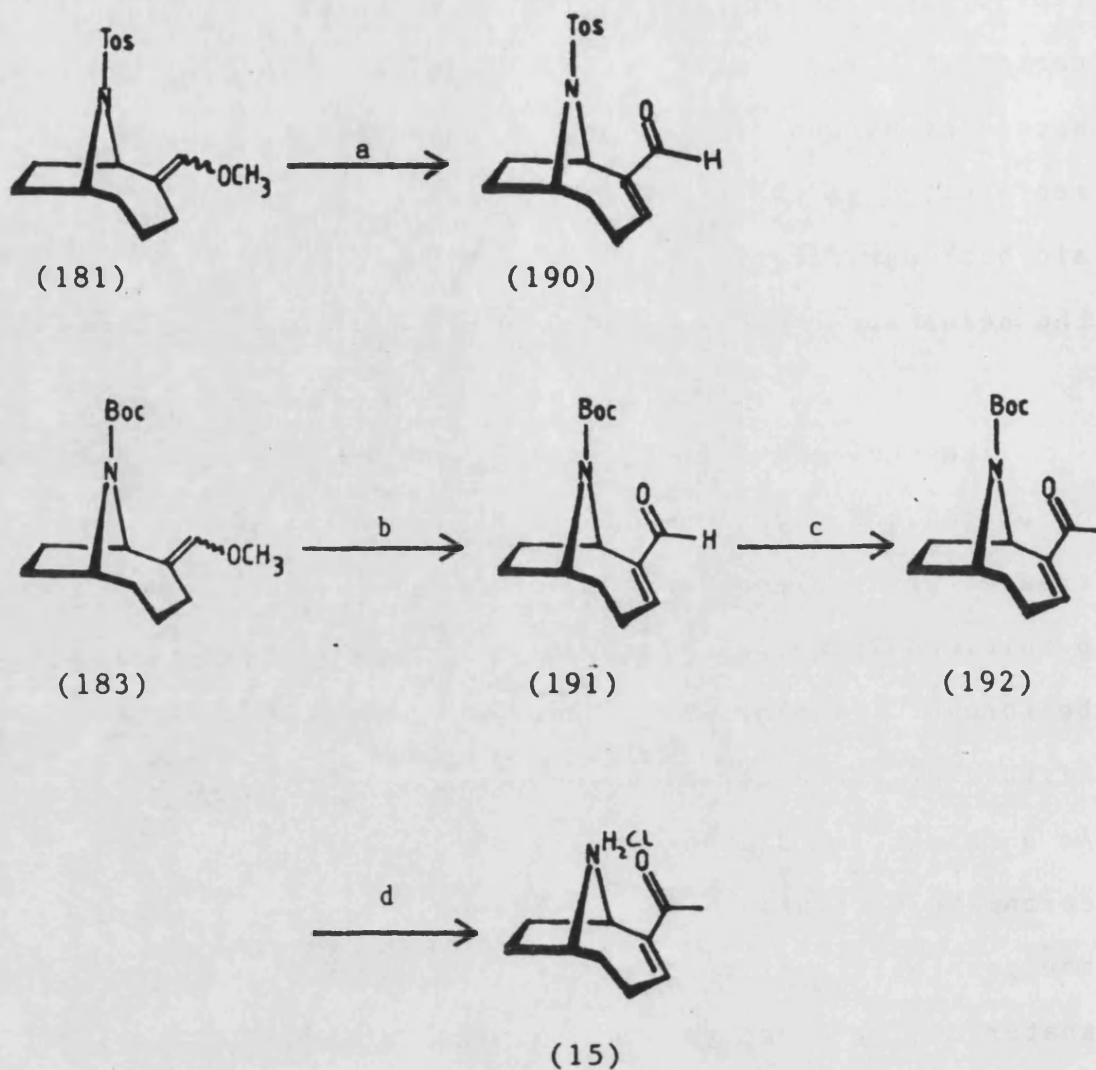


a) (i) $h\nu$, O_2 , methylene blue, (ii) $(\text{C}_6\text{H}_5)_3$; b) $(\text{CH}_3)_3\text{SiI}$, CH_3CN , 21%; c) $(\text{CH}_3)_3\text{SiI}$, CH_3CN , 65%.

SCHEME 49

(184). Sulphonamide (179) was also successfully deprotected with lithium in liquid ammonia at -78°C . The secondary amines synthesised by these reactions were reprotected as their t-butylcarbamate derivatives to aid both purification and further functionalisation of the adducts.

The enol ether (179) was irradiated in the presence of oxygen and methylene blue. This mixture was then treated with triphenylphosphine in the hope that the p-toluenesulphonamide derivative of anatoxin-a would be formed¹⁰¹ (scheme 49). However, the reaction gave a mixture of products which we were unable to identify. As a result, we did not repeat the reaction on the carbamate derivative (189). Instead, we investigated the possibility of forming derivatives of dihydroanatoxin-a by hydrolysis of the enol ethers (179) and (186). After the successful hydrolysis of enol ether (179) with trimethylsilyl iodide, to give the ketone (188), the carbamate (186) was treated in the same manner¹⁰² (scheme 49). This led to the formation of the t-butylcarbamate derivative of dihydroanatoxin-a (189) in 65% yield. The production of (189) constituted a formal synthesis of anatoxin-a as Rapoport had converted this compound into the toxin in 3 steps and 50% yield⁴².



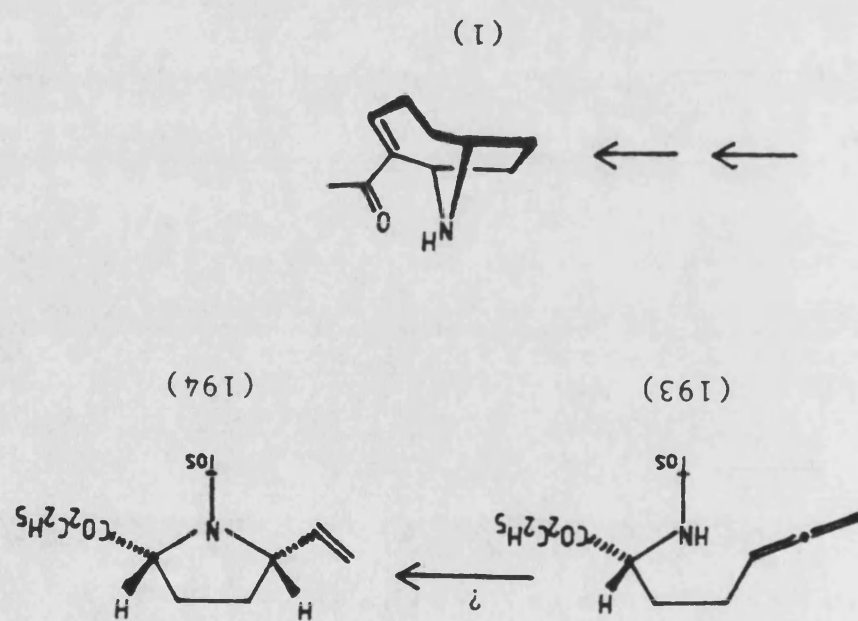
a) (i) $\text{C}_6\text{H}_5\text{SeCl}$, CH_2Cl_2 ; (ii) MCPBA, CH_2Cl_2 , 76%; b) (i) $\text{C}_6\text{H}_5\text{SeCl}$, CH_2Cl_2 ; (ii) MCPBA, CH_2Cl_2 , 61%; c) (i) CH_3MgI , $(\text{C}_2\text{H}_5)_2\text{O}$; (ii) pyridinium chlorochromate, acetone, 37%; d) 3M HCl, ethyl acetate, 95%.

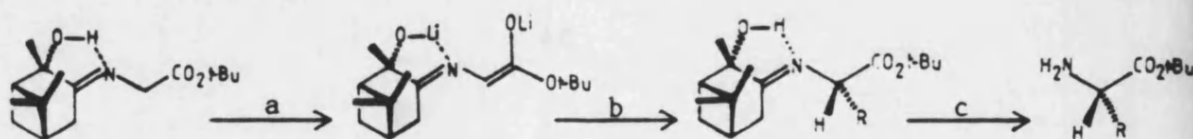
SCHEME 50

Following our success in producing a formal synthesis of anatoxin-a, we attempted to synthesise the toxin from the enol ether (183) (scheme 50). We hoped to achieve this by forming the α,β -unsaturated aldehyde (191) by applying the methodology developed by Nicolaou¹⁰⁸. Initially to test the feasibility of this methodology, the enol ether (181) was treated with phenyl selenyl chloride followed by MCPBA, to give the α,β -unsaturated aldehyde (190). After this success, carbamate (183) was treated in the same manner, and aldehyde (191) formed in 61% yield. Addition of an excess of methylmagnesium iodide to aldehyde (191), followed by oxidation of the adduct with pyridinium chlorochromate¹⁰⁹, gave the t-butylcarbamate derivative of anatoxin-a in 37% yield. It should be noted that these last two reactions were only attempted once, and on a small scale, therefore, these results were not optimised. The synthesis of anatoxin-a was completed by the acid hydrolysis of the carbamate group.

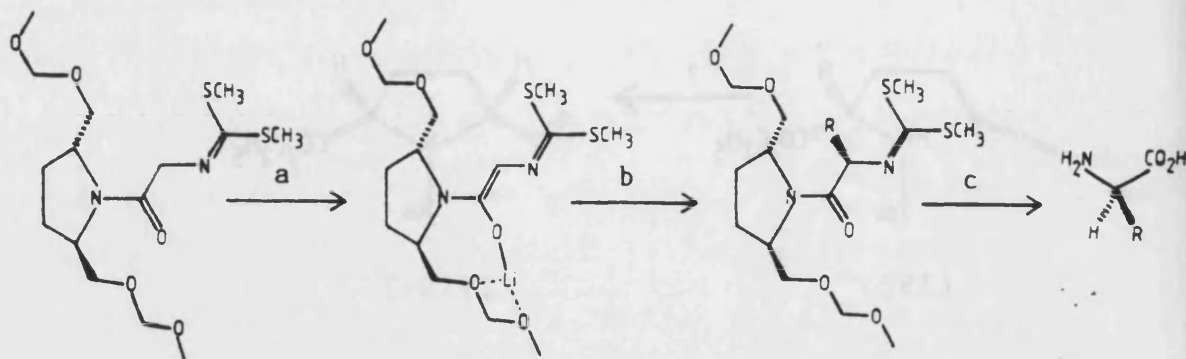
With a route to racemic anatoxin-a complete, it now remained to produce the neurotoxin enantiospecifically. We rationalised that if the pyrrolidine (194) could be synthesised in an optically pure form, then racemisation should not occur in the subsequent steps required to produce the toxin by our method. If the pyrrolidine (194) could be formed enantiospecifically by the silver-

SCHEME 51

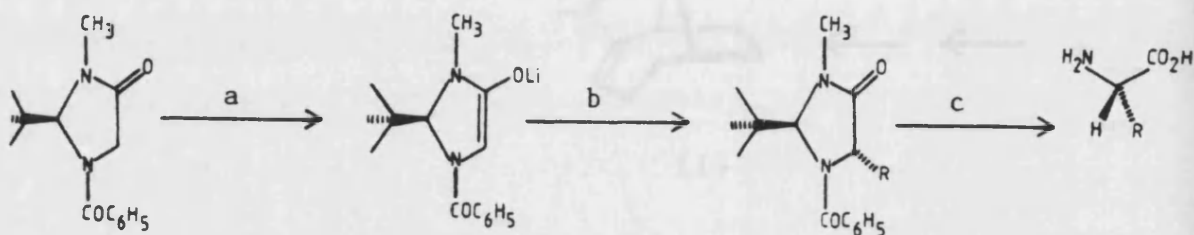




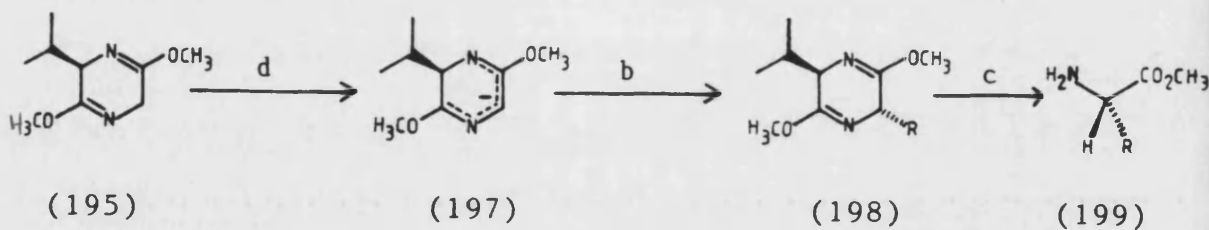
Reference 105 a.



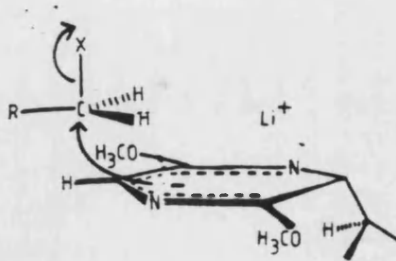
Reference 105 b.



Reference 105 c.



Reference 106.



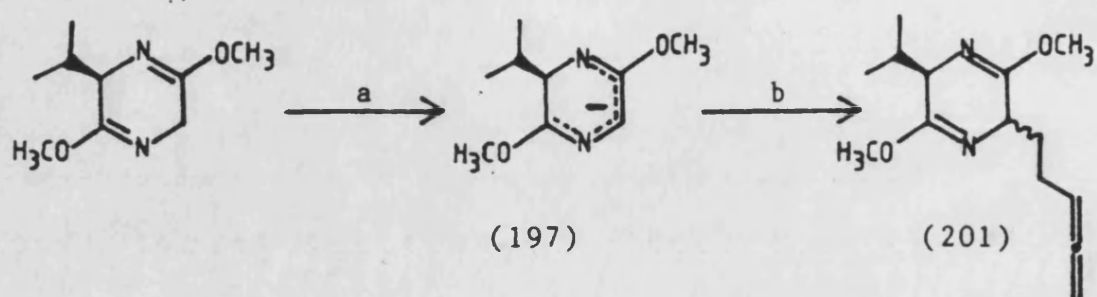
(200)

a) Lithium diisopropylamide, b) RX, c) H_3O^+ , d) N-Butyllithium.

SCHEME 52

(i) induced cyclisation of the optically pure sulphonamide (193), then the problem would be reduced to the enantiospecific formation of a glycine derivative (scheme 51). Due to the increasing importance in research of optically pure amino acids, several methods of producing optically active glycine derivatives have been reported¹⁰⁵. Some of these are outlined briefly in (scheme 52).

The method we chose to apply to this problem was that of Schollkopf, who showed that α -substituted amino acids (199) could be obtained by the "bislactim-ether method"¹⁰⁶. The bislactim-ether (195) was regiospecifically metalated with *n*-butyllithium and reacted with alkyl halides to give the adducts (198) ($\geq 95\%$ d.e.). The alkyl residue enters *trans* to the isopropyl group, thus inducing the *S* configuration at the new stereocentre. Presumably this is a result of the lithium compound being a planar dihydropyrazine anion (197), one diastereotopic side of which is strongly shielded by the relatively large isopropyl group. Schollkopf postulated that the "folded" conformation (200) represented the transition state, in which R is situated above the heterocyclic anion and thus closely approaches the centre of induction. This conformation was presumed to be stabilised by either a favourable HOMO (anion) - LUMO (R) interaction or by van der Waals attraction. Hydrolysis of the alkylated dihydropyrazines liberated the *S*-amino acid methyl

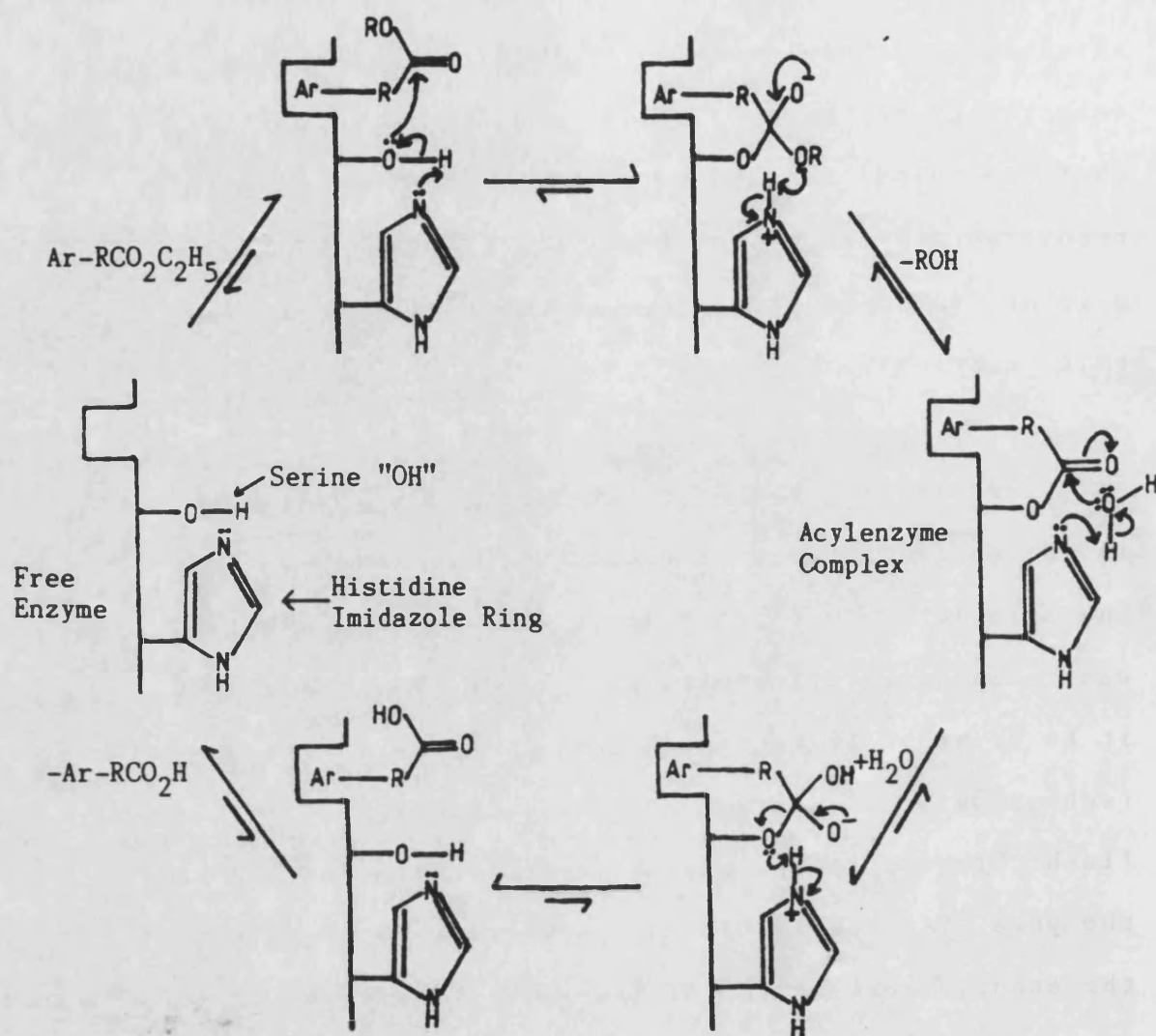


a) $n\text{-BuLi}$, THF; b) (137), THF, 81%, (trans:cis, 8:1).

SCHEME 54

esters (199). These were readily separated from the by-product, L-valine methyl ester, by distillation. Advantages of this reaction include the high diastereoselectivity of the alkylation, coupled with the fact that the chiral auxiliary (in this case L-valine) is recovered and may be re-used. Schollkopf has produced a great deal of work further extending the utility of this reaction¹⁰⁷.

The planar dihydropyrazine anion (197) was generated in THF as described by Schollkopf¹⁰⁶, and treated with the allenic iodide (137). After work up, the adduct (201) was isolated in 81% yield, but ¹H nmr analysis showed it to be a 8:1 mixture of trans- and cis-isomers respectively (scheme 54). These isomers could be separated by careful flash chromatography. As we required large amounts of the pure diastereoisomer, attempts were made to improve the enantiomeric excess of the product by altering the reaction conditions. For example (i) quenching the reaction at -78°C, (ii) addition of HMPA to the reaction mixture before the quench and (iii) conducting the reaction in DME as a solvent. These all made little difference to the product ratio. However, when the reaction was conducted at -90°C, the enantiomeric excess was improved significantly to 92%. Unfortunately, the yield of the adduct fell to a lowly 13%. Obviously the required optically pure substituted glycine



SCHEME 55

derivative could be produced by this method, but, as has already been noted, we required large amounts of the amino ester in very high e.e. It was this requirement that led us to look at the possibility of an enzymatic resolution of the substrate.

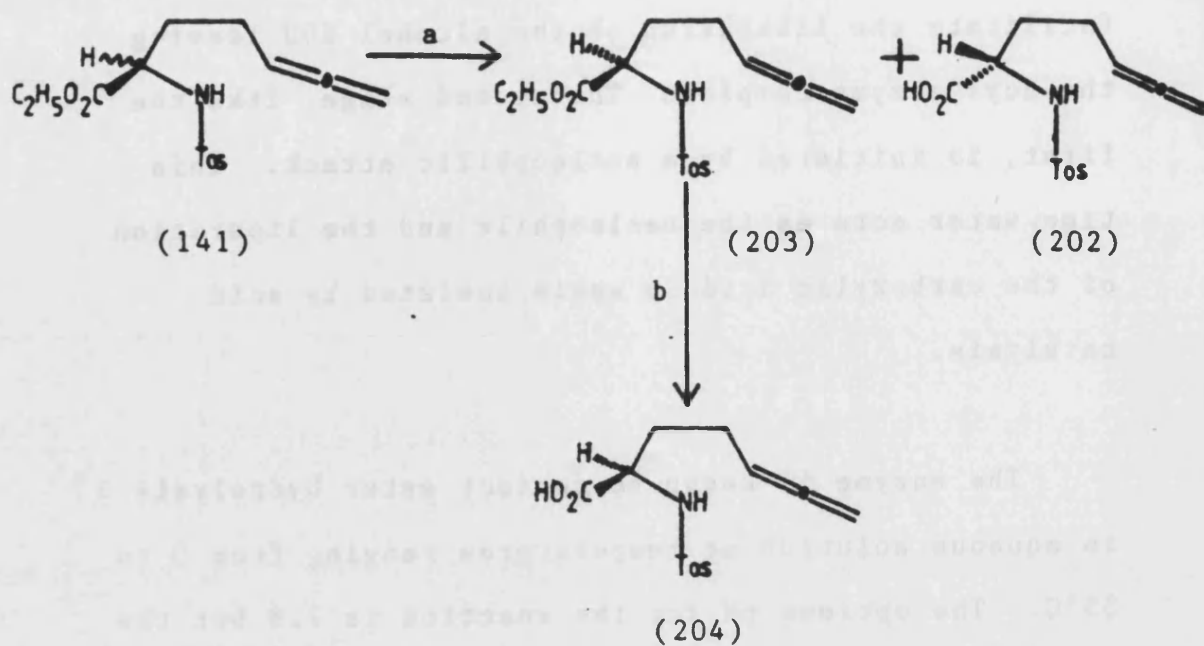
Of the numerous enzymes of the chymotrypsin family which have been identified¹⁰⁸, the inexpensive and commercially available α -chymotrypsin, isolated from bovine pancreas, has been by far the most intensively studied¹⁰⁹. In vivo this enzyme acts as a protein endopeptidase and catalyses, with great specificity, the hydrolysis of nonterminal peptide bonds that are adjacent to a phenylalanine, tyrosine or tryptophan residue. Organic chemists have conducted extensive in vitro studies on the ability of α -chymotrypsin to catalyse the hydrolysis of a broad spectrum of amides, esters and other carboxylic acid derivatives.

α -Chymotrypsin is thought to hydrolyse esters by the mechanism outlined in (scheme 55)¹¹⁰. First, the substrate attaches to a hydrophobic binding pocket at the active site, usually via an aromatic substituent. A histidine residue then acts as a base catalyst, this enables the oxygen of a serine residue to undergo nucleophilic attack on the carbonyl groups of the enzyme bound substrate. The imidazole group of the histidine residue then acts as an acid catalyst to

facilitate the liberation of the alcohol ROH leaving the acyl enzyme complex. The second stage, like the first, is initiated by a nucleophilic attack. This time, water acts as the nucleophile and the liberation of the carboxylic acid is again assisted by acid catalysis.

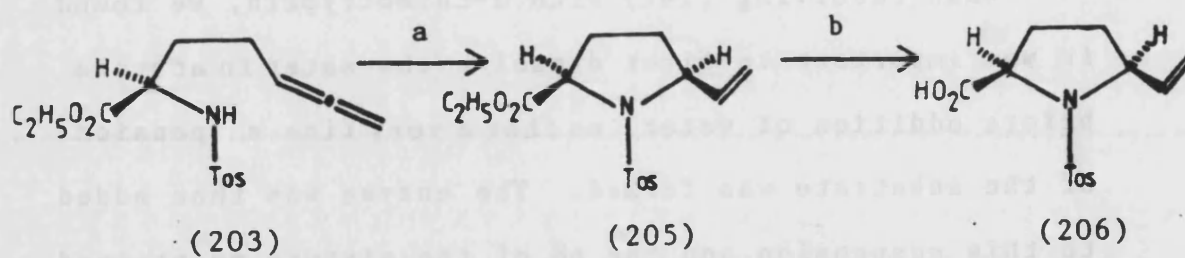
The enzyme is known to conduct ester hydrolysis in aqueous solution at temperatures ranging from 0 to 35°C. The optimum pH for the reaction is 7.8 but the reaction will proceed between pH 5.8 and pH 9.8. The addition of many organic co-solvents to the reaction mixture, to aid dissolution of the substrate is also possible. With the addition of such co-solvents, significant hydrolysis has been observed at temperatures as low as -33°C¹⁰⁹.

When resolving (141) with α -chymotrypsin, we found it was important to first dissolve the ester in acetone before addition of water, so that a very fine suspension of the substrate was formed. The enzyme was then added to this suspension and the pH of the mixture maintained at 7.2 by automatic pH-stat addition of aqueous sodium hydroxide. When alkali addition indicated 50% hydrolysis had occurred, the reaction was quenched by temporarily raising the pH of the mixture to 11 and the products isolated by standard methods¹⁰⁹. The unreacted ester (203) was shown to be a single optical isomer by a ¹H nmr



a) α -Chymotrypsin, acetone/H₂O 1:10; b) LiOH, H₂O, THF.

SCHEME 56



a) AgBF₄, CH₂Cl₂, 91%; b) LiOH, H₂O, THF, 99%.

SCHEME 57

chiral shift study (appendix 2). Similarly the acid (202) was shown to be optically pure by ^1H nmr data obtained for a mixture of (202) and (R)- α -methylbenzylamine (RMBA) (appendix 2). The resolved ester (203) was converted to the corresponding acid (204), by lithium hydroxide hydrolysis. The optical rotation of (204) proved it was the opposite optical isomer to the acid (202) (scheme 56). The absolute configuration of the resolved ester (203) was shown to be R by another worker at Bath¹¹¹.

Silver(I) catalysed cyclisation of (203) gave the cis-2,5-disubstituted pyrrolidine (205) (scheme 57). This ester was hydrolysed with lithium hydroxide to give the acid (206). The acid was shown to be a single optical isomer by the ^1H nmr data obtained for a mixture of (206) and RMBA (appendix 2).

CONCLUSION

Both a formal synthesis and a full synthesis of racemic anatoxin-a have been developed. The key step of both routes was the silver(I) induced cyclisation of a suitably substituted allenic sulphonamide to form a cis-2,5-disubstituted pyrrolidine. The exclusive formation of the required cis-isomer is thought to be largely a result of steric interactions in the proposed intermediate. However, predictions concerning the mechanism of this reaction are only tentative, as little is known about the actual transition state.

The formal synthesis gave the t-butylcarbamate derivative of dihydroanatoxin-a (189), from the N-benzylidene derivative of glycine ethyl ester, in 10 steps and an overall yield of 6.6%. Rapoport had converted (189) into the natural product in 3 steps and a yield of 50%. A sample of anatoxin-a was obtained via the full synthesis, from the same substrate, in 12 steps and an overall yield of 3.3%. It is possible that the efficiency of the latter route could be improved as the process was not fully optimised.

We also hoped to prepare anatoxin-a enantiospecifically, from an optically pure sample of the pyrrolidine substrate (204). Although we successfully synthesised the pyrrolidine as a single enantiomer, time ran out

before we could produce the natural product from this material. However, we do not believe that racemisation will occur in the subsequent steps required to form the toxin and therefore hope that this will be achieved in the near future.

RECOMMENDATIONS FOR FURTHER WORK

- 1) The full synthesis of anatoxin-a that we have developed should be fully optimised.
- 2) Different processes that may effect the final ketone homologation more efficiently than the Wittig chemistry we have employed, should be investigated.
- 3) The synthesis of anatoxin-a should be repeated with the optically pure substrate we have produced, to show that the toxin can be formed enantiospecifically by this methodology.
- 4) Various analogues of the toxin should be prepared, to enable:
 - i) the toxin to be attached to a polymer support.
 - ii) the determination of the structural features that contribute to the high activity of the toxin.
 - iii) the possible discovery of an even more potent agonist.
- 5) The mechanism of the silver(I) catalysed cyclisation of allenic amines should be investigated further.

EXPERIMENTAL

Where appropriate, solvents were dried before use in the following manner; tetrahydrofuran was distilled from sodium and benzophenone; acetonitrile, 1,2-dimethoxyethane, di(2-methoxyethyl) ether, dimethyl sulphoxide, methylene chloride and toluene were distilled from calcium hydride; diethyl ether was distilled from lithium aluminium hydride; acetone and ethyl acetate were distilled from anhydrous potassium carbonate; ammonia was distilled from sodium; all other solvents were distilled prior to use. Light petroleum refers to the fraction collected between 60 and 80°C unless otherwise stated. Brine refers to a saturated aqueous solution of sodium chloride. Reagents obtained commercially were purified by standard techniques prior to use¹¹⁴. Flash chromatography was conducted using Merck Kieselgel 60 H (Art 7736). Infrared spectra were taken on a Perkin Elmer 1310 grating spectrometer. The nuclear magnetic resonance spectra were run at 60 MHz on a Hitachi Perkin Elmer R24B, at 90 MHz on a Jeol JNM PS 90 and at 270MHz on a Jeol JNM GX 270 MHz instrument using tetramethylsilane (TMS $\delta=0$) as an internal standard in deuteriochloroform¹¹⁵. All chemical shifts were reported on the δ scale as parts per million downfield from TMS. Mass spectra were recorded on a 7070E VG Analytical Organic Mass Spectrometer. Elemental analyses

were calculated on a Carlo Erba Elemental Analyser Model 1106. Reactions were performed under an atmosphere of N_2 unless otherwise stated. Reactions were concentrated at circa 25 mmHg on a rotary evaporator. Tlc data was obtained on Merck tlc DC-Alufolien Kieselgel 60F²⁵⁴ plates. Preparative tlc was performed on commercial 1 x 200 x 200 mm glass backed SiO_2 gel plates (Merck 60F²⁵⁴). A TTT11 Titrograph fitted with a SBU syringe burette was used as an autotitrator. Optical rotations were measured on a PE141 Radiometer using a mercury lamp (254 nm).

E-Pent-2-en-4-yn-1-ol (132)

To stirred anhydrous liquid ammonia (1.5 l) was added just sufficient sodium metal (0.5 g) to produce a permanent blue colour and then anhydrous iron(III) chloride (0.5 g, 3.0 mmol). The solution was treated over 1 h with more sodium metal (49.5 g, 2.2 mole total) cut into small pieces. The reaction mixture was stirred for 2 h when a grey suspension formed which was cooled to -78°C . Acetylene gas, freed from acetone by passing it through concentrated sulphuric acid, was passed through the suspension for 4 h producing a uniformly black liquid. To this solution was added epichlorohydrin (92.5 g, 78.2 ml, 1 mole) over 2 h. During as well as for 2 h after the addition, the temperature of the solution was kept at $\leq -45^{\circ}\text{C}$. The reaction mixture was allowed to warm to -33°C and stirred vigorously for 3 h. Powdered anhydrous ammonium chloride (75 g, 1.4 mole) was added to the reaction mixture over 1 h and the ammonia allowed to evaporate. The remaining salt mass was dissolved in water (500 ml) and extracted with diethyl ether (10 x 150 ml). The extracts were combined, dried (MgSO_4) and the solvent removed by simple distillation. The residue was distilled at reduced pressure (0.5 mmHg) and the distillate collected in a single receiver

cooled to -40°C . The distillate was redistilled to give (132) as a colourless oil (34 g, 42%); b.p. 68°C at 12 mmHg (lit.⁷⁹ $65-66^{\circ}\text{C}$ at 12 mmHg); δ_{H} (60 MHz, CDCl_3) 6.2 (1H, dt, J 16, 5 Hz), 5.6 (1H, dd, J 16, 2 Hz), 4.1 (2H, broad d, J 5 Hz), 3.7 (1H, broad s), 2.9 (1H, d, J 2 Hz).

Penta-3,4-dien-1-ol (133)

To a stirred slurry of lithium aluminium hydride (8.7 g, 0.23 mole) in diethyl ether (600 ml) at 0°C was added a solution of (132) in diethyl ether (100 ml). The reaction mixture was refluxed for 8 h then cooled to 0°C and treated with sufficient aqueous sodium potassium tartrate (1.5 M) to form a colourless granular precipitate. The reaction mixture was filtered and the solid residue washed with diethyl ether (3 x 75 ml). The organic filtrates were combined, dried (Na_2SO_4) and concentrated. The residue was distilled to give the allenic alcohol (133) as a clear oil (26.1 g, 83%); b.p. $58-60^{\circ}\text{C}$ at 14 mmHg (lit.⁸⁰ $57-58^{\circ}\text{C}$ at 16 mmHg); δ_{H} (60 MHz, CDCl_3) 5.0 (1H, quintet, J 6 Hz), 4.6 (2H, dt, J 6, 3 Hz), 3.6 (2H, t, J 6 Hz), 3.1 (1H, s), 2.2 (2H, qd, J 6, 3 Hz).

1-Bromopenta-3,4-diene (134)

Bromine (24 g, 0.15 mole) was added dropwise to a solution of triphenyl phosphite (48.4 g, 0.16 mole) in diethyl ether (75 ml) at 0°C. The reaction mixture was stirred for 30 min, washed with diethyl ether (50 ml) and dried in vacuo (15 mmHg) at 50°C to give triphenyl phosphite dibromide as a pale yellow powder. This powder was cooled to -10°C and treated with a solution of the alcohol (133) (10.5 g, 0.13 mole) in pyridine (9.9 g, 0.13 mole). When the addition was complete, the reaction mixture was stirred for 3 h at ambient temperature, then diethyl ether (50 ml) and water (30 ml) added. The aqueous phase was separated and extracted with diethyl ether (4 x 25 ml). The organic extracts were combined, dried (MgSO₄), concentrated and the residue distilled to give the allenic bromide (134) as a clear oil (10.8 g, 59%); b.p. 69–70°C at 70 mmHg (lit.⁷⁹ 69–70°C at 70 mmHg); δ_{H} (60 MHz, CDCl₃) 5.0 (1H, quintet, J 6 Hz), 4.2 (2H, dt, J 6, 3 Hz) 3.3 (2H, t, J 6 Hz), 2.5 (2H, qd, J 6, 3 Hz).

Ethyl Penta-3,4-dienoate (136)

Triethyl orthoacetate (100 g, 0.62 mole, 113 ml), propargyl alcohol (26 g, 0.46 mole, 27 ml) and propionic acid (1 g, 13.5 mmol, 1 ml) were heated at 100°C for 1 h and ethanol distilled from the reaction mixture. The temperature was raised to 140°C and more distillate

collected. When distillation ceased, the temperature was raised to 180°C and the solution allowed to reflux for 4 hrs. The reaction mixture was cooled to ambient temperature and THF (75 ml) and aqueous hydrochloric acid (2 M, 50 ml) were added. The mixture was stirred for 18 h and then poured into light petroleum (b.p. 30-40°C) (75 ml). The aqueous layer was separated and the organic layer washed with saturated aqueous sodium bicarbonate (50 ml), brine (50 ml) and dried (MgSO_4). The solvents were removed by simple distillation and the residue distilled to give the allenic ester (136) as a clear oil (6.5 g, 11%); b.p. 68-71°C at 20 mmHg (lit.⁸⁰ 65-70°C at 20 mmHg); δ_{H} (60 MHz, CDCl_3) 5.2 (1H, quintet, J 7 Hz), 4.7 (2H, dt, J 7, 3 Hz), 4.1 (2H, q, J 7 Hz), 3.0 (2H, dt, J 7, 3 Hz), 1.2 (3H, t, J 7 Hz).

Penta-3,4-dien-1-ol (133)

To a stirred suspension of lithium aluminium hydride (4.8 g, 0.13 mole) in diethyl ether (250 ml) cooled to -78°C was added a solution of (136) (12.6 g, 0.10 mole) in diethyl ether (25 ml). The reaction mixture was allowed to warm to ambient temperature over 1 h and stirred for a further 1 h. The reaction mixture was cooled to -10°C and carefully treated with saturated aqueous sodium sulphate until the aluminium salts formed a colourless granular precipitate. The suspension was filtered and the solid residue washed with diethyl

ether (3 x 50 ml). The combined filtrates were dried (Na_2SO_4), concentrated by simple distillation and the residue distilled to give (133) as a clear oil (6.2 g, 74%); b.p. 58–60°C at 14 mmHg (lit.⁸⁰ 57–58°C at 16 mmHg); δ_{H} (60 MHz, CDCl_3) 5.0 (1H, quintet J 6 Hz), 4.6 (2H, dt, J 6, 3 Hz), 3.6 (2H, t, J 6 Hz), 3.1 (1H, s), 2.2 (2H, qt, J 6, 3 Hz).

1-Iodopenta-3,4-diene (137)

To a stirred solution of the allenic alcohol (133) (13.6 g, 0.16 mole) and triethylamine (24.4 g, 0.24 mole, 33.6 ml) in methylene chloride (250 ml) at -30°C was added a solution of methanesulphonyl chloride (20.2 g, 0.18 mole, 13.6 ml) in methylene chloride (70 ml). Triethylamine hydrochloride began to precipitate and the solution was stirred at -10°C for 1 h. The reaction mixture was poured into saturated aqueous sodium bicarbonate (300 ml) and the aqueous layer separated. The organic layer was washed with brine (300 ml), dried (Na_2SO_4) and concentrated to give crude 1-methanesulphonylpenta-3,4-diene as a clear oil (25.5 g, 98%). This oil was not purified further but dissolved in acetone (340 ml) and treated with anhydrous sodium iodide (48 g, 0.4 mole). This stirred suspension was heated at reflux for 16 hrs, then cooled to ambient temperature, poured into light petroleum (b.p. 30–40°C) (600 ml) and washed with saturated aqueous sodium

thiosulphate (600 ml). The organic layer was separated, washed with brine (300 ml), dried (Na_2SO_4) and the solvents removed by simple distillation. The residue was distilled to give (137) as a clear oil (21.5 g, 69%); b.p. 64–68°C at 14 mmHg (lit.⁸⁰ 66–70°C at 20 mmHg); δ_{H} (60 MHz, CDCl_3) 5.1 (1H, quintet, J 7 Hz), 4.7 (2H, dt, J 7, 3 Hz), 3.1 (2H, t, J 7 Hz), 2.5 (2H, qt, J 7, 3 Hz).

(1-Methoxyethyl)diphenylphosphine Oxide (177)

To a stirred solution of diisopropylamine (1.1 mmol, 111 mg, 154 μl) in THF (25 ml) at 0°C was added a solution of n-butyllithium in hexane (1.6 M, 1.1 mmol, 0.69 ml). After 10 min the reaction mixture was treated with a solution of the phosphine oxide (176) (246 mg, 1.0 mmol) in THF (10 ml) and a red colour formed. After 10 min at 0°C the solution was cooled to -78°C and treated with methyl iodide (170 mg, 75 μl , 1.2 mmol), the colour was lost immediately and after 5 min the reaction was quenched by addition of saturated aqueous ammonium chloride (20 ml). After warming to ambient temperature, the mixture was treated with water (30 ml) and extracted with methylene chloride (5 x 50 ml). The organic extracts were combined, dried (Na_2SO_4), concentrated and purified by careful recrystallisation to give (177) as a colourless crystalline solid (100 mg, 39%); m.p. 78–79°C (toluene - light petroleum); (lit. 77–78°C⁹⁸); δ_{H} (60 MHz, CDCl_3) 7.4–8.3 (10 H, m), 4.1 (1H, pentet, J 7 Hz), 3.3 (3H, s), 1.5 (3H, dd, J 15, 7 Hz).

N-(phenylmethylene)glycine Ethyl Ester (138)

To a stirred slurry of glycine ethyl ester hydrochloride (14.0 g, 0.1 mole) in methylene chloride (100 ml), was added a mixture of benzaldehyde (10.8 g, 10.3 ml, 0.1 mole), triethylamine (20.3 g, 28.0 ml, 0.2 mole) and anhydrous magnesium sulphate (8.3 g, 0.07 mole) in methylene chloride (100 ml). The reaction mixture was stirred at ambient temperature for 16 h, filtered and the filtrate concentrated. The residue was dissolved in diethyl ether (150 ml), washed with water (3 x 50 ml), then brine (50 ml), dried (MgSO_4) and concentrated to give (138) as a colourless oil (17.7 g, 93%); δ_{H} (60 MHz, CDCl_3) 8.1 (1H, s), 7.5-7.8 (2H, m), 7.1-7.4 (3H, m), 4.3 (2H, s), 4.1 (2H, q, J 7 Hz), 1.2 (3H, t, J 7 Hz).

N-((4-methylphenyl)sulphonyl)-1-(penta-3,4-dienyl)-glycine Ethyl Ester (141)

To a stirred suspension of freshly sublimed potassium t-butoxide (5.8 g, 0.052 mole) in THF (120 ml) at -78°C was added a solution of the N-benzylidene (138) (9 g, 0.047 mole) in THF (20 ml). After the addition was complete, the orange solution that formed was stirred for a further 10 min then treated with a solution of 1-iodopenta-3,4-diene (10 g, 0.052 mole) in THF (25 ml) and the reaction mixture allowed to warm to ambient temperature over 1.5 h. After stirring the

solution for an additional 1.5 h, (the colour of the reaction changed to dark green), the reaction mixture was cooled to 0°C and added to rapidly stirred ice cold saturated aqueous ammonium chloride (100 ml). The mixture was diluted with water (40 ml), extracted with diethyl ether (5 x 75 ml), the organic extracts combined, concentrated and the residue dissolved in diethyl ether (80 ml). The solution was stirred rapidly with aqueous hydrochloric acid (2 M, 50 ml) at ambient temperature for 40 min to effect imine hydrolysis. The organic layer was then separated, extracted with aqueous hydrochloric acid (2 M, 3 x 20 ml) and the combined aqueous phases washed with diethyl ether (20 ml). The organic phases were combined, concentrated (to 20 ml) and extracted with aqueous hydrochloric acid (2 M, 2 x 20 ml). The combined acid fractions were cooled in an ice bath and carefully treated with aqueous sodium hydroxide (20 M) to pH 11. The aqueous phase was then thoroughly extracted with methylene chloride (5 x 40 ml), the organic extracts were combined, washed with brine (50 ml), dried (Na_2SO_4) and concentrated to a yellow oil presumably crude (penta-3,4-dienyl)glycine ethyl ester. This oil was not purified further but dissolved in pyridine (40 ml), cooled to 0°C and treated with p-toluenesulphonyl chloride (9.9 g, 0.052 molè). The reaction mixture was allowed to stand at 5°C for 16 h when the pyridine was removed in vacuo. The

resulting oil was dissolved in methylene chloride (100 ml), washed with aqueous hydrochloric acid (2 M, 50 ml) and the aqueous washings extracted with methylene chloride (50 ml). The organic fractions were then combined, dried (Na_2SO_4), concentrated and purified by flash chromatography (light petroleum - ethyl acetate, 4:1) to give (141) as colourless needles (10.1 g, 66%); m.p. 45-46°C (diethyl ether - light petroleum); R_f (light petroleum - ethyl acetate, 4:1) 0.33; ν_{max} (film) 1955, 1725 cm^{-1} ; δ_{H} (270 MHz, CDCl_3) 7.73 (2H, d, part of AA'BB', J 8.1 Hz), 7.29 (2H, d, part of AA'BB', J 8.1 Hz), 5.26 (1H, broad d, J 9.3 Hz), 5.07 (1H, quintet, J 6.6 Hz), 4.68 (2H, dt, J 6.6, 3.3 Hz), 3.93 (2H, q, J 7.15 Hz), 3.90 (1H, m), 2.41 (3H, s), 2.03-2.15 (2H, m), 1.65-1.93 (2H, m), 1.10 (3H, t, J 7.14 Hz); δ_{C} (270 MHz, CDCl_3) 208.4 (C:C:C), 171.6 (CO), 143.6 (C), 136.6 (C), 129.6 (CH), 127.2 (CH), 88.4 (CH), 75.7 (CH_2), 61.6 (CH_2), 55.2 (CH), 32.6 (CH_2), 23.5 (CH_2), 21.5 (CH_3), 13.9 (CH_3); m/z (CI) 324 ($M+1$)⁺; (found: C, 59.4; H, 6.6; N, 4.4%. $\text{C}_{16}\text{H}_{21}\text{NO}_4\text{S}$ requires: C, 59.42; H, 6.54; N, 4.33%).

N-((4-methylphenyl)sulphonyl)-1-(penta-3,4-dienyl)-glycine (141 a)

A solution of allenic ester (141) (323 mg, 1 mmol) in THF (5 ml) and water (3 ml) was treated with lithium hydroxide monohydrate (158 mg, 3.8 mmol). The reaction mixture was stirred at ambient temperature for 60 h, then diluted with water (15 ml) and washed with methylene chloride (15 ml). The aqueous solution was acidified to pH 2 with aqueous hydrochloric acid (2 M) and extracted with methylene chloride (4 x 20 ml). The organic extracts were combined, dried (Na_2SO_4) and concentrated to give (141 a) as a colourless solid (170 mg, 57%); m.p. 107–109°C (benzene – light petroleum); R_f (light petroleum – ethyl acetate, 3:2) 0.10; ν_{max} (CHCl_3) 1955, 1725 cm^{-1} ; δ_{H} (270 MHz, CDCl_3) 7.35 (2H, d, part of AA'BB', J 8.1 Hz), 7.28 (2H, d, part of AA'BB', J 8.1 Hz), 5.53 (1H, broad s), 5.01 (1H, quintet, J 6.6 Hz), 4.66 (2H, dt, J 6.6, 3.3 Hz), 3.93 (1H, broad q, J 4.5 Hz), 2.40 (3H, s), 1.64–2.12 (4H, m), one proton missing due to very broad signal for O-H; δ_{C} (90 MHz, CDCl_3) 4698.5, 3980.7, 3243.4, 3078.6, 2923.58, 2867.43, 1988.52, 1710.2, 1236.6, 726.3, 528.6, 484.6; m/z (CI) 296 ($\text{M}+1$)⁺; (found: C, 57.1; H, 5.7; N, 5.1%. $\text{C}_{14}\text{H}_{17}\text{NSO}_4$ requires: C, 56.93; H, 5.80; N, 4.74%).

Cis-5-ethenyl-1-((4-methylphenyl)sulphonyl)prolineEthyl Ester (145)

A solution of the allenic ester (141) (5 g, 15.5 mmol) in methylene chloride (50 ml) was treated with silver tetrafluoroborate (300 mg, 1.54 mmol) and stirred in the dark at ambient temperature for 7 days. The reaction mixture was then washed with water (25 ml) and the aqueous washings extracted with methylene chloride (3 x 25 ml). The organic phases were combined, washed with brine (30 ml), dried (Na_2SO_4), concentrated and purified by flash chromatography (light petroleum - ethyl acetate, 5:1) to give (145) as a colourless crystalline solid (4.90 g, 98%); m.p. 58-59°C (diethyl ether - light petroleum); R_f (light petroleum - ethyl acetate, 4:1) 0.40; ν_{max} (CHCl_3) 1740, 1600 cm^{-1} ; δ_{H} (270 MHz, CDCl_3) 7.76 (2H, d, part of AA'BB', J 8.2 Hz), 7.30 (2H, d, part of AA'BB', J 8.2 Hz), 5.79 (1H, ddd, J 17.0, 10.3, 6.5 Hz), 5.37 (1H, dt, J 17.0, 1.3 Hz), 5.09 (1H, dt, J 10.1, 1.3 Hz), 4.37 (1H, t, J 6.9 Hz), 4.25 (1H, m), 4.19 (2H, qd, J 7.2, 2.6 Hz), 2.42 (3H, s), 1.93-2.05 (2H, m), 1.69-1.91 (2H, m), 1.27 (3H, t, J 7.1 Hz); δ_{C} (270 MHz, CDCl_3) 171.99 (CO), 143.51 (C), 137.99 (CH), 135.73 (C), 129.44 (CH), 127.71 (CH), 116.39 (CH_2), 63.17 (CH), 61.82 (CH), 61.32 (CH_2), 31.92 (CH_2), 29.26 (CH_2), 21.51 (CH_3), 14.05 (CH_3); m/z (CI) 324 ($M+1$)⁺; (found: C, 59.1; H, 6.4; N, 4.2%. $\text{C}_{16}\text{H}_{21}\text{NO}_4\text{S}$ requires: C, 59.42; H, 6.54; N, 4.33%).

Cis-5-ethenyl-1-((4-methylphenyl)sulphonyl)proline (148)

A solution of the ester (145) (568 mg, 1.76 mmol) in THF (8 ml) and water (5 ml) was treated with lithium hydroxide monohydrate (277 mg, 6.6 mmol). The reaction mixture was stirred at ambient temperature for 16 h then diluted with water (20 ml) and washed with methylene chloride (20 ml). The aqueous solution was acidified to pH 2 with aqueous hydrochloric acid (2 M) and extracted with methylene chloride (4 x 25 ml). The organic extracts were combined, dried (Na_2SO_4) and concentrated to give (148) as a colourless solid (375 mg, 72%); m.p. 150–151°C (benzene – light petroleum); R_f (ethyl acetate) 0.1; ν_{max} (CHCl_3) 1725 cm^{-1} ; δ_{H} (270 MHz, CDCl_3) 7.77 (2H, d, part of AA'BB', J 8.1 Hz), 7.34 (2H, d, part of AA'BB', J 8.1 Hz), 5.81 (1H, ddd, J 17.0, 10.3, 6.4 Hz), 5.35 (1H, dt, J 17.0, 1.1 Hz), 5.13 (1H, dt, J 10.3, 1.1 Hz), 4.34 (1H, dd, J 8.2, 5.6 Hz), 4.19 (1H, q, J 6.4 Hz), 1.89–2.21 (2H, m), 1.80 (2H, qd, J 6.7, 1.4 Hz), one proton missing due to very broad signal for O–H; m/z (CI) 296 ($M+1$)⁺; (found: C, 57.3; H, 5.9; N, 4.8%. $\text{C}_{14}\text{H}_{17}\text{NSO}_4$ requires: C, 56.93; H, 5.80; N, 4.74%).

Cis-5-ethenyl-1-((4-methylphenyl)sulphonyl)prolineEthyl Ester (145)

To a stirred solution of the acid (148) (40 mg, 0.14 mmol) in ethanol (5 ml) at 0°C was added acetyl chloride (1.01 g, 14 mmol, 1 ml). The reaction mixture

was stirred at 0°C for 16 h when the solvents were removed in vacuo. The residue was dissolved in methylene chloride (5 ml) and washed with water (10 ml). The aqueous washings were extracted with methylene chloride (2 x 5 ml). The organic fractions were combined, washed with brine, (5 ml), dried (Na_2SO_4) and concentrated to give (145) as a colourless solid (35 mg, 79%), the analytical data of which was identical to that for (145) which had been prepared earlier by a different method.

Cis-5-(1-deuteroethenyl)-1-((4-methylphenyl)sulphonyl)-proline Ethyl Ester (166)

A solution of allene (141) (500 mg, 1.55 mmol) in deuteriochloroform (15 ml) was treated with deuterium oxide (5 ml) and stirred at ambient temperature for 48 h. The organic phase was separated, dried (freshly roasted MgSO_4) and filtered to give a solution of (165) in deuteriochloroform which was shown by ^1H nmr to be 64% N-deuterated; δ_{H} (270 MHz, CDCl_3) 7.72 (2H, d, part of AA'BB', J 8.2 Hz), 7.29 (2H, d, part of AA'BB', J 8.2 Hz), 5.25 (0.36 H, d, J 9.3 Hz), 5.07 (1H, quintet, J 6.6 Hz), 4.69 (2H, dt, J 6.6, 3.3 Hz), 3.93 (2H, q, J 7.2 Hz), 3.90 (1H, m), 2.41 (3H, s), 2.03-2.15 (2H, m), 1.65-1.93 (2H, m), 1.10 (3H, t, J 7.1 Hz). A portion of the solution (3 ml) was treated with silver tetrafluoroborate (59 mg, 0.3 mmol) and stirred at ambient

temperature in the dark for 24 h. The reaction mixture was then washed with water (3 ml) and the aqueous washings extracted with methylene chloride (3 x 3 ml). The organic phases were combined, washed with brine (5 ml), dried (Na_2SO_4), concentrated and the residue purified by flash chromatography (light petroleum - ethyl acetate, 4:1) to give the colourless crystalline solid (166) (90 mg, 90%) which was shown by ^1H nmr to be 62% deuterated at C1 of the ethenyl substituent; δ_{H} (270 MHz, CDCl_3), 7.75 (2H, d, part of AA'BB', J 8.4 Hz), 7.30 (2H, d, part of AA'BB', J 8.4 Hz), 5.80 (0.38 H, ddd, J 17.0, 10.3, 6.5 Hz), 5.38 (0.38 H, dt, J 17.0, 1.3 Hz), 5.37 (0.62 H, s), 5.09 (0.38 H, dt, J 10.1, 1.3 Hz), 5.09 (0.62 H, s), 4.35 (1H, t, J 6.8 Hz), 4.19-4.28 (1H, m), 4.19 (2H, qd, J 7.2, 2.6 Hz), 2.42 (3H, s), 1.93-2.05 (2H, m), 1.69-1.91 (2H, m), 1.27 (3H, t, 7.2 Hz).

N-((1,1-dimethylethoxy)carbonyl)-1-(penta-3,4-dienyl)-glycine Ethyl Ester (142)

To a stirred suspension of freshly sublimed potassium t-butoxide (2.4 g, 21.4 mmol) in THF (60 ml) at -78°C was added a solution of the N-benzylidene (138) (3.7 g, 19.5 mmol) in THF (10 ml). After the addition was complete the orange solution that formed was stirred for a further 10 min at -78°C . A solution of 1-iodopenta-3,4-diene (4.2 g, 21.4 mmol) in THF (10 ml) was then

added dropwise and the reaction mixture allowed to warm to ambient temperature over 1.5 h. After stirring the solution for an additional 1.5 h (the colour changed to dark green) the reaction mixture was cooled to 0°C and added to rapidly stirred ice cold saturated aqueous ammonium chloride (50 ml). The mixture was diluted with water (20 ml) and extracted with diethyl ether (6 x 40 ml), the organic extracts were combined, concentrated and the residue dissolved in diethyl ether (40 ml). The solution was stirred rapidly with aqueous hydrochloric acid (2 M, 25 ml) at ambient temperature for 40 min to effect imine hydrolysis. The organic layer was separated, extracted with aqueous hydrochloric acid (2 M, 3 x 10 ml) and the combined aqueous phases washed with diethyl ether (10 ml). The organics were combined, concentrated (to 10 ml) and extracted with aqueous hydrochloric acid (2 M, 2 x 10 ml). The combined acid fractions were cooled in an ice bath, carefully treated with aqueous sodium hydroxide (20 M) to pH 11 and thoroughly extracted with methylene chloride (5 x 20 ml). The organic extracts were combined, washed with brine (25 ml), dried (Na_2SO_4) and concentrated to give crude 1-(penta-3,4-dienyl)glycine ethyl ester (140) (a yellow oil). This was not purified further but dissolved in methylene chloride (50 ml), treated with di-*t*-butyl dicarbonate (4.3 g, 19.5 mmol) and stirred at ambient temperature for 16 h. The reaction mixture was then heated at reflux

for 2 h, cooled to ambient temperature, treated with water (30 ml) and extracted with methylene chloride (5 x 30 ml). The organic extracts were combined, dried (Na_2SO_4), concentrated and the residue purified by flash chromatography (light petroleum - ethyl acetate, 9:1) to give (142) as a colourless crystalline solid (2.6 g, 49%); m.p. 52–54°C (diethyl ether - light petroleum); R_f (light petroleum - ethyl acetate, 4:1) 0.45; ν_{max} (CHCl_3) 1960, 1730, 1705 cm^{-1} ; δ_{H} (270 MHz, CDCl_3) 5.12 (1H, quintet, J 6.6 Hz), 5.06 (1H, broad m), 4.70 (2H, dt, J 6.6, 3.3 Hz), 4.32 (1H, broad m), 4.20 (2H, q, J 7.14 Hz), 1.86–2.14 (3H, m), 1.73 (1H, m), 1.45 (9H, s), 1.28 (3H, t, J 7.14 Hz); m/z (CI) 270 ($M+1$)⁺; (found 196.0957 ($M - \text{OC}_4\text{H}_9$)⁺. $\text{C}_{10}\text{H}_{14}\text{NO}_3$ requires 196.0974); (found: C, 62.6; H, 8.8; N, 5.2%. $\text{C}_{14}\text{H}_{23}\text{NO}_4$ requires: C, 62.43; H, 8.61; N, 5.20%).

1-((1,1-Dimethylethoxy)carbonyl)-5-ethenylproline

Ethyl Ester (146)

To a solution of the allenic ester (142) (2.5 g, 9.3 mmol) in methylene chloride (50 ml) was added silver tetrafluoroborate (450 mg, 2.3 mmol) and the reaction mixture stirred in the dark at ambient temperature for 72 h. The reaction mixture was then washed with water (25 ml) and the aqueous washings extracted with methylene chloride (3 x 25 ml). The organic phases were combined, washed with brine (30 ml), dried (Na_2SO_4) and concentrated.

The residue was purified by flash chromatography (light petroleum - ethyl acetate, 4:1) to give (146) as a colourless oil (2.2 g, 88%); R_f (light petroleum - ethyl acetate, 4:1) 0.60; ν_{max} (film) 1730, 1690 cm^{-1} ; δ_{H} (270 MHz, CDCl_3) 5.83 (1H, m), 5.34 (1H, m) 5.09 (1H, m), 4.10-4.62 (2H, m), 4.19 (2H, qd, J 6.8, 2.8 Hz), 1.62-2.32 (4H, m), 1.42 (9H, broad s), 1.29 (3H, t, J 6.8 Hz); m/z (CI) 270 (M+1)⁺; (found: m/z 196.1299 (M-CO₂-C₂H₅)⁺. C₁₁H₁₈NO₂ requires 196.1337).

Cis-5-(2-hydroxyethyl)-1-((4-methylphenyl)sulphonyl)-proline Ethyl Ester (168)

To a stirred solution of boron trifluoride etherate (350 mg, 300 μl , 2.4 mmol) in di(2-methoxyethyl) ether (2 ml) was added a solution of sodium borohydride (1 M, 1.0 ml, 1.0 mmol) in di(2-methoxyethyl) ether and the diborane generated was bubbled in a stream of nitrogen through a solution of alkene (145) (200 mg, 0.62 mmol) in THF (5 ml) at 20°C. After stirring at ambient temperature for 20 min, the reaction mixture was cooled to 0°C and carefully treated with sufficient water to destroy the excess diborane. To the solution was first added aqueous sodium hydroxide (3 M, 0.3 ml, 0.9 mmol), followed by hydrogen peroxide (30%, 0.3 ml, 0.9 mmol), taking care that the temperature of the reaction does not rise above 25°C. The reaction mixture was then stirred at ambient temperature for 30 min after which

water (10 ml) and ethyl acetate (10 ml) were added. This mixture was extracted with ethyl acetate (5 x 10 ml) and the organic extracts combined, dried (Na_2SO_4), concentrated and the residue purified by flash chromatography (light petroleum - ethyl acetate, 3:2) to give alcohol (168) (167 mg, 79%) as a colourless glass; R_f (light petroleum - ethyl acetate, 1:1) 0.21; ν_{max} (CHCl_3) 3520, 1735 cm^{-1} ; δ_{H} (270 MHz, CDCl_3) 7.75 (2H, d, part of AA'BB', J 8.2 Hz), 7.35 (2H, d, part of AA'BB', J 8.2 Hz), 4.21 (2H, q, J 7.1 Hz), 4.11 (1H, t, J 8.3 Hz), 4.09 (1H, m), 3.78 (1H, dt, J 11.9, 4.4 Hz), 2.44 (3H, s), 1.89–2.12 (2H, m), 1.73–1.80 (2H, m), 1.61 (1H, m), 1.41 (1H, m), 1.29 (3H, t, J 7.1 Hz), 1.05–1.23 (1H, m), 3.0 (broad signal, possibly OH); δ_{C} (270 MHz, CDCl_3) 172.26 (CO), 144.08 (C), 134.16 (C), 129.19 (CH), 127.48 (CH), 61.77 (CH), 61.56 (CH_2), 58.77 (CH_2), 58.76 (CH), 37.58 (CH_2), 30.69 (CH_2), 29.38 (CH_2), 21.53 (CH_3), 14.04 (CH_3); m/z (CI) 342 ($M+1$)⁺; this compound was never produced sufficiently pure to obtain a satisfactory elemental analysis.

Cis-5-(2-bromoethyl)-1-((4-methylphenyl)sulphonyl)proline
Ethyl Ester (173)

A solution of bromine (2 M.) in THF was added dropwise to a stirred solution of triphenylphosphine (73 mg, 0.28 mmol) in THF (3 ml) at 0°C. A colourless precipitate immediately began to form. When just enough bromine

solution had been added to give the reaction a permanent yellow colour a further portion of triphenylphosphine (~5 mg) was added so as the colour was removed. The reaction mixture was then stirred at ambient temperature for 10 min before being cooled to 0°C and treated with a solution of (168) (63.5 mg, 0.19 mmol) in THF (1 ml). The reaction was stirred for 10 min, then allowed to warm to ambient temperature, when water (2 ml) was added and the mixture extracted with ethyl acetate (5 x 5 ml). The organic extracts were combined, washed with brine (10 ml), dried (Na_2SO_4), concentrated and purified by flash chromatography (ethyl acetate - light petroleum, 1:3) to give (173) as a colourless oil (67.3 mg, 90%); R_f (ethyl acetate - light petroleum, 1:1) 0.73; ν_{max} (CHCl_3) 1740 cm^{-1} ; δ_{H} (270 MHz, CDCl_3) 7.76 (2H, d, J 8.1 Hz), 7.33 (2H, d, J 8.1 Hz), 4.24 (1H, t, J 7.2 Hz), 4.21 (2H, qd, J 7.1, 1.3 Hz), 3.92 (1H, quintet, J 6.2 Hz), 3.40-3.66 (2H, m), 2.44 (3H, s), 2.43 (1H, hex, J 7.1 Hz), 1.93 (2H, t, J 7.3 Hz), 1.63 (2H, q, J 7.0 Hz), 1.30 (3H, t, J 7.1 Hz), 2.06 (1H, m); m/z (CI) 404, 406 ($M+1$)⁺; This compound was never produced sufficiently pure to obtain a satisfactory elemental analysis.

1-(Cis-5-(2-hydroxyethyl)-1-((4-methylphenyl)sulphonyl) - pyrrolidin-2-yl)-2-(methylsulphonyl)ethanone (169)

A solution of dimethylsulphone (1.36, 14.5 mmol) in THF (100 ml) at 0°C was treated with a solution of *n*-butyllithium in hexane (1.6 M, 8.1 ml, 12.9 mmol) and

a colourless precipitate formed. This suspension was stirred at ambient temperature for 20 min, then cooled to -10°C and treated with a solution of (168) (1.1 g, 3.2 mmol) in THF (25 ml). The reaction mixture rapidly cleared and after 10 min was quenched by addition of saturated aqueous ammonium chloride (40 ml). The mixture was acidified to pH 4 with aqueous hydrochloric acid (2 M), diluted with water (30 ml) and extracted with ethyl acetate (5 x 50 ml). The organic extracts were combined, washed first with water (50 ml) followed by brine (70 ml), then dried (Na_2SO_4) and concentrated to a colourless oil. This oil was purified by flash chromatography (ethyl acetate - light petroleum, 4:1) to give (169) (0.79 g, 63%); R_f (ethyl acetate - light petroleum, 4:1) 0.30; ν_{max} (film) 3570, 1740 cm^{-1} ; δ_{H} (400 MHz, CDCl_3) 7.70 (2H, d, part of AA'BB', J 8.0 Hz), 7.36 (2H, d, part of AA'BB', J 8.0 Hz), 4.60 (1H, dq, J 15.2, 1.1 Hz), 4.35 (1H, dt, J 15.2, 0.5 Hz), 3.94–4.70 (4H, m), 3.77 (1H, broad m), 3.14 (3H, q, J 0.5 Hz), 2.45 (3H, s), 2.15 (1H, m), 1.83–1.94 (2H, m), 1.77 (1H, m), 1.64 (1H, m), 1.41 (1H, m); δ_{C} (90 MHz, CDCl_3) 198.50 (CO), 144.86 (C), 132.67 (C), 130.18 (CH), 127.85 (CH), 69.18 (CH), 60.57 (CH_2), 59.59 (CH), 59.05 (CH_2), 42.15 (CH_3), 38.52 (CH_2), 30.55 (CH_2), 26.98 (CH_2), 21.56 (CH_3); m/z (CI) 390 ($M+1$)⁺; this compound was never produced sufficiently pure to obtain a satisfactory elemental analysis.

1-(Cis-5-(2-bromoethyl)-1-((4-methylphenyl)sulphonyl)-pyrrolidin-2-yl)-2-(methylsulphonyl)ethanone (170)

A solution of bromine (2 M) in THF was added dropwise to a stirred solution of triphenylphosphine (440 mg, 1.68 mmol) in THF (15 ml) at 0°C. A colourless precipitate immediately began to form. When just enough bromine solution had been added to give the reaction a permanent yellow colour a further portion of triphenylphosphine (~20 mg) was added so as the colour was removed. The reaction mixture was then stirred at ambient temperature for 10 min before being cooled to 0°C and treated with a solution of (169) (365 mg, 0.94 mmol) in THF (5 ml). After 10 min the reaction mixture was allowed to warm to ambient temperature when water (20 ml) was added and the mixture extracted with ethyl acetate (5 x 20 ml). The organic extracts were combined, washed with brine (20 ml), dried (Na₂SO₄), concentrated and purified by flash chromatography (ethyl acetate - light petroleum, 3:2) to give (170) as colourless needles (331 mg, 78%); m.p. 123°C (ethyl acetate - light petroleum); R_f (ethyl acetate - light petroleum, 4:1) 0.64; ν_{\max} (CHCl₃) 1720 cm⁻¹; δ_{H} (400 MHz, CDCl₃) 7.73 (2H, d, part of AA'BB', J 8.1 Hz), 7.38 (2H, d, part of AA'BB', J 8.1 Hz), 4.59 (1H, dq, J 15.3, 1.5 Hz), 4.36 (1H, d, J 15.3 Hz), 4.09 (1H, t, J 8.0 Hz), 3.85 (1H, tdd, J 8.0, 5.6, 3.6 Hz), 3.62 (1H, dt, J 10.4, 6.0 Hz), 3.40 (1H, ddd, J 10.4, 8.6, 5.6 Hz), 3.14 (3H, s), 2.51 (1H,

ddt, J 14.4, 8.8, 5.6 Hz), 2.45 (3H, s), 2.14 (1H, dddd, J 18.0, 9.6, 8.0, 6.8 Hz), 2.02 (1H, ddt, J 14.4, 8.0, 5.6 Hz), 1.80 (1H, dtd, J 13.2, 7.2, 4.4 Hz), 1.50–1.67 (2H, m); m/z (CI) 452, 454 (M+1)⁺; (found: C, 42.4; H, 4.8; N, 3.0%. C₁₆H₂₂NS₂O₅Br requires C, 42.48; H, 4.90; N, 3.09%).

9-((4-Methylphenyl)sulphonyl)-3-(methylsulphonyl)-9-azabicyclo-[4.2.1]nonan-2-one (171)

To a stirred solution of the bromide (170) (725 mg, 1.6 mmol) in dry dimethyl sulphoxide (30 ml) was added sodium hydride (99%, 155 mg, 6.4 mmol) and the reaction mixture stirred at 40°C for 2 h. The solution was then poured onto a rapidly stirred mixture of aqueous hydrochloric acid (2 M, 40 ml) and ice (20 g). After 10 min the suspension was filtered, the solid collected and dried in vacuo. This solid was crystallised from methanol to give (171) (152 mg) as a colourless solid. The filtrate was extracted with methylene chloride (5 x 25 ml) and the extracts combined with the mother liquor from the crystallisation, dried (Na₂SO₄), concentrated and purified by flash chromatography (ethyl acetate - light petroleum, 4:1) to give (171) (344 mg). Total yield of (171) (496 mg, 83%), m.p. 215°C (methanol); R_f (ethyl acetate - light petroleum, 4:1) 0.39; ν_{\max} 1735 cm⁻¹; δ_{H} (400 MHz, CDCl₃) 7.70 (2H, d, part of AA'BB', J 8.5 Hz), 7.33 (2H, d, part of AA'BB', J 8.5 Hz), 4.56 (1H, dd, J

12.0, 2.0 Hz), 4.53 (1H, m), 4.45 (1H, dd, J 10.0, 2.0 Hz), 3.05 (3H, s), 2.66 (1H, dtd, J 14.0, 4.0, 1.5 Hz), 2.44 (3H, s), 2.28 (1H, tt, J 7.0, 4.0 Hz), 1.65–2.00 (5H, m), 1.55 (1H, m); m/z (CI) 372 ($M+1$)⁺; (found: C, 51.4; H, 5.6; N, 3.6%. $C_{16}H_{21}NS_2O_5$ requires C, 51.73; H, 5.70; N, 3.77%). Continued elution gave *cis*-9-((4-methylphenyl)sulphonyl)-2-((methylsulphonyl)methylene)-3-oxa-9-azabicyclo[4.2.1]nonane (172) as a colourless solid (15 mg, 3%); m.p. 240–242°C (decomp) (methanol); R_f (ethyl acetate – light petroleum, 4:1) 0.20; ν_{max} ($CHCl_3$) 1620cm^{-1} ; δ_H (400 MHz, $CDCl_3$) 7.72 (2H, d, part of AA'BB', J 8.2 Hz), 7.31 (2H, d, part of AA'BB', J 8.2 Hz), 5.60 (1H, s), 4.63 (1H, dd, J 11.0, 3.0 Hz), 4.45–4.51 (1H, m), 4.37 (1H, dt, J 13.0, 4.0 Hz), 4.12 (1H, td, J 13.0, 2.0 Hz), 3.00 (3H, s), 2.43 (3H, s), 2.19–2.30 (2H, m), 1.97 (1H, m), 1.77 (1H, m), 1.61–1.69 (2H, m); nOe data : irradiation at δ 5.6 resulted in a 9% enhancement of the signal at δ 4.63; m/z (EI) 371 (M)⁺; (found: m/z 371.0874 (M)⁺. $C_{16}H_{21}NS_2O_5$ requires (M)⁺ 371.0860).

9-((4-Methylphenyl)sulphonyl)-9-azabicyclo[4.2.1]nonan-2-one (174)

Aluminium foil (4 cm^2) was submerged in an aqueous solution of mercuric chloride (0.074 M) for 15 sec, then washed with absolute ethanol followed by diethyl ether. The freshly treated foil was added to an unstirred solution of the keto sulphone (171) (46 mg, 0.12 mmol)

in THF (3 ml) and water (0.3 ml) at 60°C. Further portions of freshly treated aluminium foil (4 cm²) were added to the reaction every 30 min. After 3 h the reaction was filtered through a pad of celite and the solid residue washed with ethyl acetate (5 x 5 ml). The organic filtrates were combined, dried (Na₂SO₄), concentrated and purified by flash chromatography (ethyl acetate - light petroleum, 1:1) to give (174) as a colourless solid (33.4 mg, 92%); m.p. 155°C (methanol); R_f (ethyl acetate - light petroleum, 1:1) 0.62; ν_{max} (nujol) 1715 cm⁻¹; δ_{H} (400 MHz, CDCl₃) 7.71 (2H, d, part of AA'BB', J 8.5 Hz), 7.30 (2H, d, part of AA'BB', J 8.5 Hz), 4.51 (1H, dq, J 12.0, 3.0 Hz), 4.32 (1H, dd, J 10.0, 3.0 Hz), 3.05 (1H, td, J 14.0, 2.4 Hz), 2.43 (3H, s), 2.32 (1H, dd, J 14.0, 6.0 Hz), 2.12 (1H, tt, J 14.0, 4.0 Hz), 1.50-1.90 (7H, m); m/z (CI) 294 (M+1)⁺; (found: C, 61.3; H, 6.6; N, 4.4%. C₁₅H₁₉NSO₃ requires C, 61.41; H, 6.53; N, 4.77%).

(E+Z)-2-(1-Methoxyethylidene)-9-((4-methylphenyl)-sulphonyl)-9-azabicyclo[4.2.1]nonane (179)

To a stirred solution of diisopropylamine (56.6 mg, 78 μ l, 0.56 mmol) in DME (2 ml) at -50°C was added a solution of n-butyllithium in hexane (1.6 M, 330 μ l, 0.53 mmol). The reaction mixture was stirred for 10 min, cooled to -78°C and treated with a solution of phosphine oxide (177) (146 mg, 0.56 mmol) in DME (2 ml). Immediately a deep red colour formed, and after 20 min the

reaction mixture was treated with a solution of the ketone (174) (41 mg, 0.14 mmol) in DME (2 ml). The solution was stirred for 5 min then quenched with saturated aqueous ammonium chloride (4 ml) and the colour was lost. The mixture was warmed to ambient temperature, diluted with water (2 ml) and extracted with ethyl acetate (5 x 5 ml). The organic extracts were combined, dried (Na_2SO_4) and concentrated to a colourless solid. This solid was dissolved in THF (5 ml), treated with sodium hydride (99%, 34 mg, 1.4 mmol) and stirred at ambient temperature for 14 h. The reaction mixture was cooled to 0°C, carefully quenched with water (3 ml) and extracted with ethyl acetate (5 x 5 ml). The organic extracts were combined, dried (Na_2SO_4), concentrated and the residue purified by flash chromatography (ethyl acetate - light petroleum, 2:3) to give (179) as a colourless solid (43.6 mg, 97%). The enol ether (179) was a mixture of E- and Z-isomers (E:Z, 3:4). A small amount of this mixture was purified by preparative TLC (light petroleum - ethyl acetate, 4:1) and the two isomers isolated in pure form. Z-(179); R_f (light petroleum - ethyl acetate 2:1) 0.57; ν_{max} (CHCl_3) 1666, 1600 cm^{-1} ; δ_{H} (400 MHz, CDCl_3) 7.72 (2H, d, part of AA'BB', J 8.0 Hz), 7.26 (2H, d, part of AA'BB', J 8.0 Hz), 5.07 (1H, d, J 9.0 Hz), 4.35 (1H, m), 4.0 (3H, s), 2.40 (3H, s), 2.17-2.33 (2H, m), 1.70-1.85 (2H, m), 1.77 (3H, t, J 1.2 Hz), 1.25-1.60 (6H, m); m/z (EI) 335 (M)⁺; (found: m/z 335.1593 (M)⁺. $\text{C}_{18}\text{H}_{25}\text{NSO}_3$ requires (M)⁺ 335.1553).

E-(179); Rf (light petroleum - ethyl acetate, 2:1) 0.55;
 ν_{\max} (CHCl_3) 1666, 1600cm^{-1} ; δ_{H} (400 MHz, CDCl_3) 7.72
 (2H, d, part of AA'BB', J 8.5 Hz), 7.27 (2H, d, part of
 AA'BB', J 8.5 Hz), 4.77 (1H, d, J 9.0 Hz), 4.35-4.41
 (1H, m), 3.45 (3H, s), 2.87 (1H, dd, J 15.0, 8.0 Hz),
 2.41 (3H, s), 1.72-2.01 (3H, m), 1.78 (3H, d, J 2.0 Hz),
 1.46-1.68 (6H, m); m/z (EI) 335 (M)⁺; (found: m/z
 335.1609 (M)⁺. $\text{C}_{18}\text{H}_{25}\text{NSO}_3$ requires (M)⁺ 335.1553).

(α + β)-2-Acetyl-9-((4-methylphenyl)sulphonyl)-9-azabicyclo-
[4.2.1]nonane (188)

To a stirred solution of sodium iodide (9 mg,
 0.06 mmol) and (179) (10 mg, 0.03 mmol) in acetonitrile
 (1 ml) at ambient temperature was added trimethylsilyl
 chloride (6.5 mg, 0.06 mmol, 7.6 μl). The solution,
 which was stirred for 5 min, became yellow and slightly
 cloudy. The reaction mixture was quenched with aqueous
 sodium thiosulphate (1 M, 3 ml) and extracted with ethyl
 acetate (5 x 3 ml). The organic extracts were combined,
 washed with brine (5 ml), dried (Na_2SO_4), concentrated and
 the residue purified by preparative tlc to give α -(188) as a
 colourless solid (2 mg, 21%); Rf (light petroleum - ethyl
 acetate, 3:1) 0.29; δ_{H} (270 MHz, CDCl_3) 7.72 (2H, d, part
 of AA'BB', J 8.2 Hz), 7.30 (2H, d, part of AA'BB', J 8.2 Hz),
 4.55 (1H, m), 4.25 (1H, m), 3.01 (1H, m), 2.43 (3H, s),
 2.19 (3H, s), 1.22-2.03 (10 H, m). β -(188) (4.5 mg, 47%);
 Rf (light petroleum - ethyl acetate, 3:1) 0.16; δ_{H}
 (270 MHz, CDCl_3) 7.72 (2H, d, part of AA'BB', J 8.0 Hz),

7.28 (2H, d, part of AA'BB', J 8.0 Hz), 4.53 (1H, d, J 9.2 Hz), 4.32 (1H, m), 2.42 (3H, s), 2.28 (3H, s), 2.24 (1H, m), 1.20–2.08 (10H, m). ($\alpha+\beta$)-(188) ν_{\max} (CHCl₃) 1702, 1605 cm⁻¹; m/z (EI) 321 (M)⁺. A satisfactory elemental analysis or a high resolution accurate mass were not obtained for this sample. However, the reaction was not repeated on a larger scale in order for this data to be obtained as this was only a test system. The results were encouraging and the reaction was repeated on the real system.

(E+Z)-2-(1-Methoxyethylidene)-9-azabicyclo[4.2.1]nonane-9-carboxylic Acid 1,1-Dimethylethyl Ester (186)

To freshly distilled liquid ammonia (5 ml) was added a solution of the enol ether (179) (12 mg, 0.04 mmol) in THF (1 ml). The stirred solution was cooled at -78°C, treated with lithium metal (~20 mg) and after 3 min, when a permanent blue colour formed, quenched with solid ammonium acetate (50 mg) (colour lost). The ammonia was allowed to evaporate as the mixture warmed to ambient temperature. The residue was diluted with water (5 ml), made alkaline (pH 10) with aqueous sodium hydroxide (3 M) and extracted with chloroform (5 x 5 ml). The organic extracts were combined, dried (Na₂SO₄) and concentrated to a colourless oil. This crude oil was dissolved in methanol (2 ml), treated with di-*t*-butyl dicarbonate (25 mg, 0.11 mmol)

and stirred for 16 h. The reaction mixture was then diluted with diethyl ether (4 ml), first washed with aqueous phosphoric acid (0.2 M, 2 ml) and then saturated aqueous sodium hydrogen carbonate (2 ml). The aqueous washings were extracted with diethyl ether (5 x 2 ml) and the organic phases combined, dried (Na_2SO_4) and concentrated to a colourless oil. The crude oil was purified by flash chromatography (light petroleum - ethyl acetate, 4:1) to give (186) as a clear oil (5.5 mg, 55%); R_f (light petroleum - ethyl acetate, 2:1) 0.6; ν_{max} (CHCl_3) 1660 cm^{-1} ; δ_{H} (270 MHz, CDCl_3) 4.97 ($\frac{1}{2}\text{H}$, m), 4.69 ($\frac{1}{2}\text{H}$, m), 4.31 (1H, m) 3.48 (3H, m), 2.93 ($\frac{1}{2}\text{H}$, m), 1.81 (3H, broad s), 1.42 (9H, m), 1.30-2.40 ($9\frac{1}{2}\text{H}$, m). This ^1H nmr data was extremely complex due to the sample not only being a mixture of diastereoisomers, but also being subject to amide resonance. The sample was not characterised further but hydrolysed and the resultant product characterised more fully.

(α + β)-2-Acetyl-9-azabicyclo[4.2.1]nonane-9-carboxylic Acid 1,1-Dimethylethyl Ester (189)

To a stirred solution of sodium iodide (4.1 mg, 0.03 mmol) and the enol ether (186) (5.0 mg, 0.02 mmol) in acetonitrile (0.5 ml) at ambient temperature was added trimethylsilyl chloride (2.9 mg, 0.03 mmol, 3.5 μl). The solution, which was stirred for 5 min, became yellow

and slightly cloudy. The reaction mixture was quenched with aqueous sodium thiosulphate (1 M, 2 ml) and extracted with ethyl acetate (5 x 2 ml). The organic extracts were combined, washed with brine (4 ml), dried (Na_2SO_4), concentrated and the residue purified by flash chromatography yielding the clear oil (189) as a mixture of α and β isomers (3.5 mg, 65%, $\alpha:\beta, 2:5$). α -(189) Rf (light petroleum - ethyl acetate, 2:1) 0.46; ν_{max} (CHCl_3) 1677 cm^{-1} ; δ_{H} (270 MHz, CDCl_3) 4.64 ($\frac{1}{2}\text{H}$, m), 4.53 ($\frac{1}{2}\text{H}$, m), 4.28 ($\frac{1}{2}\text{H}$, t, J 7.9 Hz), 4.19 ($\frac{1}{2}\text{H}$, t, J 7.9 Hz), 3.20 ($\frac{1}{2}\text{H}$, dt, J 9.0, 4.5 Hz), 2.99 ($\frac{1}{2}\text{H}$, m), 2.19 ($1\frac{1}{2}\text{H}$, s), 2.16 ($1\frac{1}{2}\text{H}$, s), 1.51 ($4\frac{1}{2}\text{H}$, s), 1.48 ($4\frac{1}{2}\text{H}$, s), 1.27-2.25 (10H, m). β -(189) Rf (light petroleum - ethyl acetate, 2:1) 0.39; ν_{max} 1675 cm^{-1} ; δ_{H} (270 MHz, CDCl_3) 4.59 ($\frac{1}{2}\text{H}$, d, J 4.7 Hz), 4.49 ($\frac{1}{2}\text{H}$, d, J 9.1 Hz), 4.38 ($\frac{1}{2}\text{H}$, m), 4.22 ($\frac{1}{2}\text{H}$, m), 2.30 ($1\frac{1}{2}\text{H}$, s), 2.22 ($1\frac{1}{2}\text{H}$, s), 1.41 ($4\frac{1}{2}\text{H}$, s), 1.45 ($4\frac{1}{2}\text{H}$, s), 1.35-2.55 (11H, m). ($\alpha+\beta$)-(189) m/z (EI) 267 (M)⁺; (found: m/z 267.1818 (M)⁺. $\text{C}_{15}\text{H}_{25}\text{NO}_3$ requires (M)⁺ 267.1833). The data obtained for (189) agrees with the information reported by Rapoport for the same compound⁴¹.

(E+Z)-2-(Methoxymethylene)-9-((4-methylphenyl)sulphonyl)-9-azabicyclo[4.2.1]nonane (181)

To a stirred solution of diisopropylamine (29 mg, 0.29 mmol, 40 μl) in DME (2 ml) at -50°C was added a solution of n-butyllithium in hexane (1.6 M, 24 mmol,

150 μ l). The mixture was stirred for 10 min, cooled to -78°C and treated with a solution of phosphine oxide (176) (94 mg, 0.38 mmol) in DME (2 ml). Immediately an orange colour formed and after 20 min the reaction mixture was treated with a solution of the ketone (174) (28 mg, 0.10 mmol) in DME (2 ml). The reaction mixture was stirred for 5 min, quenched with saturated aqueous ammonium chloride (4 ml) where upon the colour was lost. The mixture was warmed to ambient temperature, diluted with water (2 ml) and extracted with ethyl acetate (5 x 5 ml). The organic extracts were combined, dried (Na_2SO_4) and concentrated to a colourless solid. This solid was dissolved in THF (5 ml), treated with sodium hydride (99%, 24 mg, 1.0 mmol) and stirred at ambient temperature for 14 h. The reaction mixture was then cooled to 0°C , quenched with water (3 ml) and extracted with ethyl acetate (5 x 5 ml). The organic extracts were combined, dried (Na_2SO_4), concentrated and the residue purified by flash chromatography (ethyl acetate - light petroleum, 2:3) to give the colourless solid (181) as a 2:3 mixture of E- and Z-isomers (29 mg, 95%); R_f (ethyl acetate - light petroleum, 1:1) 0.56; ν_{max} (CHCl_3) 1668, 1600 cm^{-1} ; δ_{H} (400 MHz, CDCl_3) 7.73 (1.2H, d, part of AA'BB', J 8.1 Hz), 7.71 (0.8H, d, part of AA'BB', J 8.3 Hz), 7.26 (1.2H, d, part of AA'BB', J 8.1 Hz), 7.23 (0.8H, d, part of AA'BB', J 8.3 Hz), 5.88 (0.4H, d, J 2.1 Hz), 5.74 (0.6H, t, J 1.7 Hz), 4.90 (0.6H, broad

d, J 6.1 Hz), 4.54 (0.4H, dd, J 10.0, 2.4 Hz), 4.38 (0.6H, m), 4.31 (0.4H, m), 3.56 (1.2H, s), 3.53 (1.8H, s), 2.56 (0.4H, dd, J 15.9, 6.1 Hz), 2.40 (3H, s), 2.29 (0.6H, t, J 13.1 Hz), 1.25–2.00 (9H, m); m/z (EI) 321 (M)⁺; (found: m/z 321.1396 (M)⁺. $C_{17}H_{23}NSO_3$ requires (M)⁺ 321.1390).

(E+Z)-2-(Methoxoxymethylene)-9-azabicyclo[4.2.1]nonane
(182)

A stirred solution of (181) (10 mg, 0.03 mmol) in toluene (3 ml) was treated with a solution of sodium bis(2-methoxyethoxy)aluminium hydride in toluene (3.4 M, 0.12 mmol, 35 μ l) and heated at reflux for 22 h. The reaction mixture was cooled to ambient temperature, treated with aqueous sodium hydroxide (3 M, 2 ml) and extracted with diethyl ether (5 x 2 ml). The organic extracts were combined, washed first with aqueous sodium hydroxide (3 M, 2 ml), then water (3 ml) and finally brine (3 ml) before being extracted with aqueous hydrochloric acid (2 M, 5 x 3 ml). The acid extracts were combined, cooled to 0°C, basified (pH 11) by addition of aqueous sodium hydroxide (20 M) and extracted with diethyl ether (5 x 5 ml). The organic extracts were combined, washed with brine (10 ml), dried (Na_2SO_4), concentrated and the residue purified by flash chromatography (chloroform – methanol – triethylamine, 85:14:1) to give a mixture of E-and Z-

isomers of the amine (182) as a brown oil (3 mg, 58%);
 Rf (chloroform - methanol - triethylamine, 80:19:1)
 0.33, 0.43; ν_{\max} 3380, 1668, 1602 cm^{-1} ; δ_{H} (270 MHz,
 CDCl_3) 6.00 ($\frac{1}{2}\text{H}$, s), 5.84 ($\frac{1}{2}\text{H}$, s), 4.79 ($\frac{1}{2}\text{H}$, d, J 6.1
 Hz), 4.38 ($\frac{1}{2}\text{H}$, d, J 6.1 Hz), 4.25 ($\frac{1}{2}\text{H}$, m), 4.16 ($\frac{1}{2}\text{H}$, m)
 3.61 ($1\frac{1}{2}\text{H}$, s), 3.57 ($1\frac{1}{2}\text{H}$, s), 1.00–2.40 (11H, m); m/z
 (EI) 167 (M)⁺; The compound proved to be unstable and
 attempts to obtain a high resolution accurate mass for
 (182) failed.

(E+Z)-2-(methoxymethylene)-9-azabicyclo[4.2.1]nonane
(182)

To freshly distilled liquid ammonia (7 ml) was
 added a solution of the enol ether (181) (8 mg, 0.025
 mmol) in THF (1 ml). The stirred solution was cooled
 to -78°C , treated with lithium metal (~ 20 mg) and after
 1 min, when a permanent blue colour formed, quenched
 with solid ammonium acetate (50 mg) (colour lost).
 The ammonia was allowed to evaporate as the reaction
 mixture warmed to ambient temperature. The residue was
 diluted with water (3 ml), made alkaline (pH 11) with
 aqueous sodium hydroxide (3 M) and extracted with
 methylene chloride (5 x 3 ml). The organic extracts
 were combined, washed with brine (5 ml), dried (Na_2SO_4)
 and concentrated. The residue was purified by flash
 chromatography (chloroform - methanol - triethylamine,
 85:14:1) to give the brown oil (182) as a mixture of
 E- and Z- isomers (2.5 mg, 60%). The data for (182)

prepared by this reaction was identical to that obtained for a sample of the amine prepared by the previous method.

(E+Z)-2-(Methoxymethylene)-9-azabicyclo[4.2.1]nonane-9-carboxylic Acid 1,1-Dimethylethyl Ester (183)

To freshly distilled liquid ammonia (30 ml) was added a solution of the enol ether (181) (61 mg, 0.19 mmol) in THF (15 ml). The stirred solution was cooled to -78°C , treated with lithium metal (~ 100 mg), and after 15 min, when a permanent blue colour formed, quenched with solid ammonium acetate (200 mg) (colour lost). The ammonia was allowed to evaporate as the reaction mixture warmed to ambient temperature. The residue was diluted with water (15 ml), made alkaline (pH 11) with aqueous sodium hydroxide (3 M) and extracted with chloroform (5 x 15 ml). The organic extracts were combined, dried (Na_2SO_4) and concentrated to a colourless oil. This crude oil was dissolved in methanol (20 ml), treated with di-*t*-butyl dicarbonate (124 mg, 0.57 mmol) and stirred for 16 h. The reaction mixture was then diluted with diethyl ether (30 ml), first washed with aqueous phosphoric acid (0.2 M, 10 ml) and then saturated aqueous sodium hydrogen carbonate (10 ml). The aqueous washings were combined and extracted with diethyl ether (5 x 20 ml). The organic fractions were combined, dried (Na_2SO_4) and concentrated to a colour-

less oil. The crude oil was purified by flash chromatography (light petroleum - ethyl acetate, 4:1) to give the clear oil (183) as a mixture of isomers (43 mg, 85 %); R_f (light petroleum - ethyl acetate, 9:1) 0.34, 0.29; ν_{\max} (CHCl_3) 1670 cm^{-1} ; δ_{H} (270 MHz, CDCl_3) 5.89 (1H, m), 4.90 ($\frac{1}{2}$ H, m), 4.38 ($\frac{1}{2}$ H, m), 4.30 (1H, m), 3.55 (3H, m), 1.45 (9H, m), 1.20-2.62 (10H, m); m/z (EI) 267 (M)⁺; (found: m/z 267.1833 (M)⁺. $\text{C}_{15}\text{H}_{25}\text{NO}_3$ requires 267.1833).

2-Carboxaldehyde-9-((4-methylphenyl)sulphonyl)-9-azabicyclo[4.2.1]non-2-ene (190)

To a stirred solution of the enol ether (181) (21.5 mg, 0.07 mmol) in methylene chloride (2 ml) at -78°C was added phenyl selenyl chloride (14.1 mg, 0.07 mmol). The reaction mixture was stirred for 2 h then treated with a solution of MCPBA (85%, 16.5 mg, 0.08 mmol) in methylene chloride (91 ml). After 30 min the reaction was quenched with water (4 ml), allowed to warm to room temperature, treated with aqueous sodium hydroxide (3 M, 0.5 ml) and saturated aqueous sodium bisulphite (0.25 ml). The mixture was extracted with methylene chloride (5 x 2 ml) and the organic extracts combined, washed with brine (5 ml), dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography (light petroleum - ethyl acetate, 3:2) to give (190) as a colourless crystalline solid (15.6 mg,

76%); m.p. 193–195°C (decomp) (methanol); R_f (light petroleum – ethyl acetate, 2:1) 0.14; ν_{max} (CHCl₃) 1680 cm⁻¹; δ_{H} (400MHz, CDCl₃) 9.28 (1H, s), 7.73 (2H, d, part of AA'BB', J 8.2 Hz), 7.27 (2H, d, part of AA'BB', J 8.2 Hz), 6.68 (1H, dd, J 6.4, 4.9 Hz), 5.07 (1H, d, J 8.2 Hz), 4.50 (1H, m), 2.75 (1H, dq, J 18.1, 5.9 Hz), 2.50 (1H, ddt, J 18.1, 9.0, 4.6 Hz), 2.41 (3H, s), 2.20 (1H, ddt, J 14.1, 9.1, 4.6 Hz), 1.73–1.83 (2H, m), 1.68 (1H, m), 1.43–1.59 (2H, m); m/z (EI) 305 (M)⁺; (found: m/z 305.1080. C₁₆H₁₉NSO₃ required 305.1084); (found: C, 62.6; H, 6.2; N, 4.3%. C₁₆H₁₉NSO₃ requires: C, 62.93; H, 6.27; N, 4.59%).

2-Carboxaldehyde-9-azabicyclo[4.2.1]non-2-ene-9-carboxylic Acid 1,1-Dimethylethyl Ester (191)

A stirred solution of the enol ether (183) (40 mg, 0.15 mmol) in methylene chloride (4 ml) at -30°C was treated with a solution of phenylselenenyl chloride (32 mg, 0.16 mmol) in methylene chloride (1 ml). After 30 min the reaction mixture was cooled to -78°C and treated with a solution of MCPBA (90%, 34.5 mg, 0.18 mmol) in methylene chloride (1 ml). The reaction mixture was stirred for 10 min, quenched with water (5 ml), allowed to warm to ambient temperature when aqueous sodium hydroxide (3 M, 0.25 ml) and saturated sodium bisulphite (0.25 ml) were added. The mixture was extracted with methylene chloride (5 x 5 ml) and the organic extracts

combined, washed with brine (5 ml), dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography (light petroleum - ethyl acetate, 2:1) to give (191) as a colourless oil (23 mg, 61%); Rf (light petroleum - ethyl acetate, 4:1) 0.14; ν_{max} (CHCl_3) 1665cm^{-1} ; δ_{H} (270 MHz, CDCl_3) 9.38 (1H, s), 9.34 (1H, s), 6.72 (1H, t, J 7.8 Hz), 6.64 (1H, t, J 5.3 Hz), 5.10 (1H, m), 5.03 (1H, d, 8.6 Hz), 4.45 (1H, broad m), 4.33 (1H, m), 2.49–2.62 (2H, m), 2.02–2.33 (3H, m), 1.55–1.76 (3H, m), 1.42 (2H, s), 1.37 (6H, s); m/z (EI) 251 (M^+); (found: m/z 251.1641. $\text{C}_{14}\text{H}_{21}\text{NO}_3$ requires 251.1521).

2-Acetyl-9-azabicyclo[4.2.1]non-2-ene-9-carboxylic Acid
1,1-Dimethylethyl Ester (192)

To a stirred solution of aldehyde (191) (11 mg, 0.04 mmol) in diethyl ether (4 ml) at -78°C was added a solution of methyl magnesium iodide (~ 0.1 mmol) in diethyl ether (1 ml). The reaction mixture was allowed to warm to -40°C over 10 min and then quenched with saturated aqueous ammonium chloride (5 ml). After warming to ambient temperature this mixture was extracted with ethyl acetate (5 x 5 ml), the organic extracts were combined, dried (Na_2SO_4) and concentrated. The residue was dissolved in methylene chloride (2.5 ml) and treated with anhydrous sodium acetate (16.5 mg, 0.2 mmol) and

pyridinium chlorochromate (43 mg, 0.2 mmol). The reaction mixture was stirred for 30 min at ambient temperature, then diluted with methylene chloride (20 ml), filtered through flourisil and concentrated. The residue was purified by flash chromatography (light petroleum - ethyl acetate, 2:1) to give (192) as a colourless oil (3.3 mg, 37%); Rf (light petroleum - ethyl acetate, 2:1) 0.33; ν_{\max} (CHCl₃) 1685, 1665, 1395 cm⁻¹; δ_{H} 6.82 (1H, t, J 5.6 Hz), 5.08-5.20 (1H, m), 4.23-4.45 (1H, m), 2.28 (3H, s), 1.95-2.60 (5H, m), 1.50-1.80 (3H, m) 1.36 (4½H, s), 1.24 (4½H, s). The data obtained for (192) correlates with that reported for the same compound by Rapoport⁴¹.

2-Acetyl-9-azabicyclo[4.2.1]non-2-ene Hydrochloride (15)

A solution of hydrochloric acid in ethyl acetate (3M) was prepared by dissolving concentrated aqueous hydrochloric acid (2 ml) in ethyl acetate (6 ml).

The t-butyl carbamate (192) (1 mg, 0.004 mmol) was dissolved in a solution of hydrochloric acid in ethyl acetate (3M, 0.5 ml) and stirred at ambient temperature for 2 h. The reaction mixture was then concentrated and purified by flash chromatography (chloroform - methanol, 9:1) to give (15) as a colourless oil (0.72 mg, 95%); Rf (chloroform - methanol, 9:1) 0.09; ν_{\max} (CHCl₃) 1602, 1640, 1673, 1713 cm⁻¹; δ_{H} (270 MHz, CDCl₃) 7.18

(1H, dd, J 7.3, 3.9 Hz), 5.26 (1H, d, J 9.0 Hz), 4.39 (1H, m), 2.38 (3H, s), 2.30-2.75 (5H, m), 1.80-2.05 (3H, m); the signals for the two protons attached to nitrogen were not detected for (15), possibly as a result of these signals being both broad and weak. The data obtained for (15) correlates with the results reported by Rapoport for the same compound⁴¹.

(2R,5S)-2,5-Dihydro-3,6-dimethoxy-2-(1-methylethyl)-5-(penta-3,4-dienyl)pyrazine (201)

To a solution of (R)-2,5-dihydro-3,6-dimethoxy-2-(1-methylethyl)pyrazine (92 mg, 0.5 mmol, 89 μ l) in THF (2 ml) at -78°C was added a solution of n-butyllithium in hexane (1.6 M, 0.55 mmol, 0.34 ml) and a yellow colour formed. The reaction mixture was stirred for 10 min, then treated with a solution of allene (137) (97 mg, 0.5 mmol) in THF (2 ml) and after 6 h at -78°C quenched with saturated aqueous ammonium chloride (2 ml). The mixture was warmed to ambient temperature, diluted with water (1 ml) and the organic solvents removed in vacuo. The aqueous residue was extracted with diethyl ether (5 x 2 ml) and the organic extracts combined, dried (MgSO_4), concentrated and purified by flash chromatography (light petroleum - ethyl acetate, 25:1) to give (201) as a clear oil (85 mg, 68%); b.p. 150°C at 0.5 mmHg; R_f (light petroleum - ethyl acetate, 1:10) 0.69; ν_{max} (film) 1960, 1687 cm^{-1} ; δ_{H} (270 MHz, CDCl_3) 5.12 (1H,

quintet, J 6.6 Hz), 4.66 (2H, dt, J 6.6, 3.3 Hz), 4.05 (1H, m), 3.94 (1H, m), 3.69 (3H, s), 3.68 (3H, s), 2.27 (1H, septet d, J 6.8, 3.3 Hz) 1.82–2.07 (3H, m), 1.80 (1H, m), 1.05 (3H, d, J 6.8 Hz), 0.69 (3H, d, J 6.8 Hz); m/z (CI) 251 (M+1)⁺; (found: C, 66.1; H 9.1; N, 11.0%. C₁₄H₂₂N₂O₂ required C, 67.17; H, 8.86; N, 11.19%).

Further elution gave a mixture of (2R, 5S)-(201) and (2R, 5R)-(201) (5:12 respectively) as a clear oil (7.5 mg, 6%). This mixture was distinguished from the first product by ¹H nmr; δ_H (270 MHz, CDCl₃) 5.14 (1H, m), 4.67 (2H, m), 4.20 (1H, m), 3.94 (1H, m), 3.68 (6H, m), 1.52–2.33 (5H, m), 1.07 (2.13 H, d, J 6.9 Hz), 1.04 (0.87 H, d, J 6.8 Hz), 0.73 (2.13 H, d, J 6.9 Hz), 0.69 (0.87 H, d, J 6.8 Hz).

(2R, 5S)-2,5-dihydro-3,6-dimethoxy-2-(1-methylethyl)-5-(penta-3,4-dienyl)pyrazine (201)

To a stirred solution of (R)-2,5-dihydro-3,6-dimethoxy-2-(1-methylethyl)pyrazine (46 mg, 0.25 mmol, 45 μl) in THF (2 ml) at -90°C was added a solution of n-butyllithium in hexane (1.6 M, 0.28 mmol, 0.17 ml) and a yellow colour formed. The reaction mixture was stirred for 10 min, then treated with a solution of allene (137) (49 mg, 0.25 mmol) in THF (1 ml) and after 2 h at -90°C quenched with saturated aqueous ammonium chloride (2 ml). The mixture was warmed to ambient temperature, diluted with water (1 ml) and the organic

solvents removed in vacuo. The aqueous residue was extracted with diethyl ether (5 x 2 ml). The combined extracts were dried (MgSO_4), concentrated and purified by flash chromatography (light petroleum - ethyl acetate, 25:1) to give (201) as a clear oil (8.3 mg, 13%). The spectral data for this sample was identical to that for the sample of (2R, 5S)-(201) produced by the previous method. However, ^1H nmr showed this sample of (210) to be contaminated with 4% of the (2R, 5R) isomer; δ_{H} (270 MHz, CDCl_3) 5.14 (1H, m), 4.67 (2H, m), 4.20 (1H, m), 3.94 (1H, m), 3.68 (6H, m), 1.52-2.33 (5H, m), 1.07 (0.12H, d, J 6.9 Hz), 1.04 (2.88H, d, J 6.8 Hz), 0.73 (0.12H, d, J 6.9 Hz), 0.69 (2.88, d, J 6.8 Hz).

(1R)-N-((4-Methylphenyl)sulphonyl)-1-(penta-3,4-dienyl)
glycine Ethyl Ester (203)^{III}

To a stirred solution of the (\pm) ester (141) in acetone (85 ml) was slowly added water (750 ml) and a very fine suspension formed. The suspension was treated with α -chymotrypsin I (25 mg) and the pH of the reaction mixture kept at 7.2 by the automatic pH stat addition of aqueous sodium hydroxide (1.05 M). After 8 h when 0.5 molar equivalents of alkali had been added the pH of the reaction mixture was raised to 11 in order to denature the enzyme. The mixture was then neutralised by addition of aqueous hydrochloric acid (2 M) and lyophilised. The residue was suspended in aqueous

sodium hydroxide (3 M, 400 ml) and extracted with diethyl ether (3 x 200 ml). The combined organic extracts were washed with brine (200 ml), dried (Na_2SO_4) and concentrated to give the resolved ester (203) as a colourless crystalline solid (480 mg, 48%). The alkaline aqueous layer was cooled to 0°C , acidified with concentrated aqueous hydrochloric acid and extracted with diethyl ether (3 x 200 ml). The combined organic extracts were washed with brine (200 ml), dried (Na_2SO_4) and concentrated to give the resolved acid (202) as a colourless crystalline solid (410 mg, 45%). (203): m.p. $50.1-50.3^\circ\text{C}$ (diethyl ether - light petroleum); $[\alpha]_{\text{D}}^{20} -39.8^\circ$ (CHCl_3 , c 35 mg ml^{-1} , l 0.1 dm); Rf, I.R. and ^1H nmr data the same as for the unresolved ester (141). The ester (203) was shown to be purely one isomer by a chiral shift study employing europium tris(3-(heptafluoropropylhydroxymethylene)-d-camphorato (see appendix 2). (202): m.p. $109.9-110.2^\circ\text{C}$ (ethyl acetate - light petroleum); $[\alpha]_{\text{D}}^{20} 21^\circ$ (CHCl_3 , c 28.2 mg ml^{-1} , l 0.1 dm); Rf, I.R. and ^1H nmr data the same as for the unresolved acid (141 a). The acid (202) was shown to be purely one isomer by the ^1H nmr data obtained for a mixture of (202) and R-methylbenzylamine (see appendix 2).

(1R)-N-((4-Methylphenyl)sulphonyl)-1-(penta-3,4-dienyl)-glycine (204)

A solution of the resolved ester (203) (25.0 mg, 0.08 mmol) in THF (2 ml) and water (0.5 ml) was treated with lithium hydroxide monohydrate (12.8 mg, 0.30 mmol). The reaction mixture was stirred at ambient temperature for 16 h then treated with aqueous hydrochloric acid (2 M, 5 ml) and extracted with diethyl ether (5 x 5 ml). The organic extracts were combined, dried (Na_2SO_4) and concentrated to give the acid (204) as a colourless crystalline solid (15.3 mg, 67%); m.p. 109.5°C (ethyl acetate - light petroleum); $[\alpha]_D^{20}$ -20° (CHCl_3 , c 30.6 mg ml^{-1} , l 0.1 dm); Rf, I.R. and ^1H nmr data the same as for the unresolved acid (141 a). (204) was shown to be purely one isomer by the ^1H nmr data obtained for a mixture of (204) and R-methylbenzylamine, this also showed (204) to be the opposite optical isomer to (202) (see appendix 2).

Cis-(2R, 5S)-1-((4-methylphenyl)sulphonyl)-5-ethenyl-proline (206)

A solution of the resolved allenic ester (203) (13.2 mg, 0.04 mmol) in methylene chloride (5 ml) was treated with silver tetrafluoroborate (20 mg, 0.1 mmol) and stirred in the dark at ambient temperature for 14 h. The reaction mixture was then washed with water (5 ml) and the aqueous washing extracted with methylene chloride

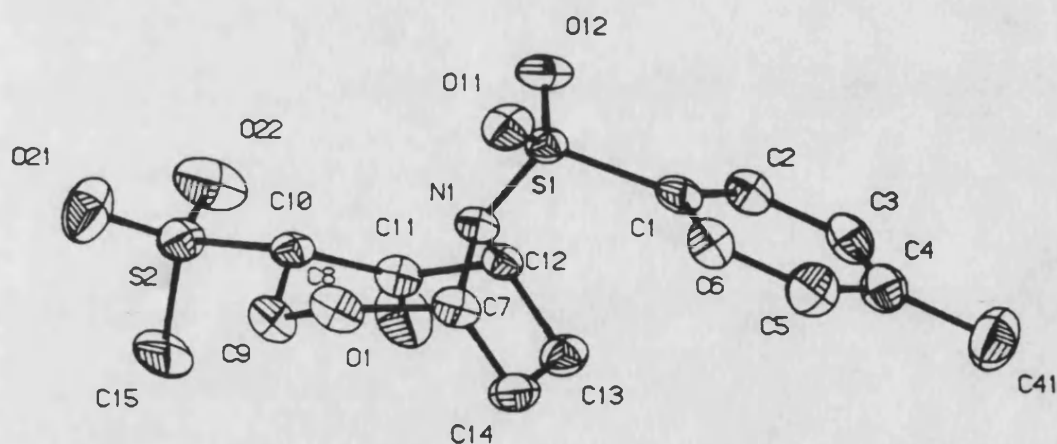
(3 x 3 ml). The organic phases were combined, washed with brine (5 ml), dried (Na_2SO_4) and concentrated to give (204) as a colourless crystalline solid (12 mg, 91%). This sample of (205) was then dissolved in THF (2 ml) and water (0.5 ml) and treated with lithium hydroxide monohydrate (8.0 mg, 0.19 mmol). The reaction mixture was stirred at ambient temperature for 14 h then treated with aqueous hydrochloric acid (2 M, 5 ml) and extracted with diethyl ether (5 x 5 ml). The organic extracts were combined, dried (Na_2SO_4) and concentrated to give the acid (205) as a colourless crystalline solid. (10.9 mg, 99%). (205): m.p. 60°C (diethyl ether - light petroleum); $[\alpha]_{\text{D}}^{20}$ 38.3° (CHCl_3 , c 24 mg ml^{-1} , l 0.1 dm); Rf, I.R. and ^1H nmr data the same as for the unresolved ester (145). (206): m.p. 151°C (ethyl acetate - light petroleum); $[\alpha]_{\text{D}}^{20}$ 9.1° (CHCl_3 , c 22 mg ml^{-1} , l 0.1 dm); Rf, I.R. and ^1H nmr data the same as for the unresolved acid (148). (206) was shown to be purely one isomer by the ^1H nmr data obtained for a mixture of (206) and R-methylbenzylamine (see appendix 2).

APPENDIX 1

The structure of the bicyclic keto sulphone (171) was determined by X-ray structural analysis¹¹³. Below is tabulated the information obtained by this technique for the sample.

Molecular Formula: $C_{16}H_{21}NO_5S_2$
 Crystal System: Monoclinic
 Cell Dimensions: $a = 12.095(2) \text{ \AA}$
 $b = 6.406(1) \text{ \AA}$
 $c = 11.260(2) \text{ \AA}$
 $\beta = 97.94 (1)^\circ$
 $v = 864.096 \text{ \AA}^3$
 Space Group: Pc
 Z: 2

The final R-factor was 3.65%.



SCHEME 58

Bond lengths (Å) for $C_{16}H_{21}NO_5S_2$

O(11)-S(1)	1.438(5)	O(12)-S(1)	1.432(4)
N(1)-S(1)	1.510(6)	C(1)-S(1)	1.971(7)
O(21)-S(2)	1.606(7)	O(22)-S(2)	1.484(6)
C(10)-S(2)	1.786(7)	C(15)-S(2)	1.607(7)
C(7)-N(1)	1.528(8)	C(12)-N(1)	1.400(6)
C(11)-O(1)	1.111(6)	C(2)-C(1)	1.512(9)
C(6)-C(1)	1.358(8)	C(3)-C(2)	1.527(10)
C(4)-C(3)	1.366(8)	C(41)-C(4)	1.668(11)
C(5)-C(4)	1.512(11)	C(6)-C(5)	1.538(11)
C(8)-C(7)	1.692(10)	C(14)-C(7)	1.571(10)
C(9)-C(8)	1.397(8)	C(10)-C(9)	1.555(9)
C(11)-C(10)	1.719(9)	C(12)-C(11)	1.529(9)
C(13)-C(12)	1.567(9)	C(14)-C(13)	1.599(11)

Bond angles (deg.) for $C_{16}H_{21}NO_5S_2$

O(12)-S(1)-O(11)	121.3(3)	N(1)-S(1)-O(11)	93.7(3)
N(1)-S(1)-O(12)	110.9(3)	C(1)-S(1)-O(11)	116.5(3)
C(1)-S(1)-O(12)	103.4(3)	C(1)-S(1)-N(1)	110.8(3)
O(22)-S(2)-O(21)	122.0(4)	C(10)-S(2)-O(21)	116.3(3)
C(10)-S(2)-O(22)	101.9(4)	C(15)-S(2)-O(21)	102.8(4)
C(15)-S(2)-O(22)	112.8(4)	C(15)-S(2)-C(10)	99.0(4)
C(7)-N(1)-S(1)	122.3(4)	C(12)-N(1)-S(1)	106.3(4)
C(12)-N(1)-C(7)	112.0(4)	C(2)-C(1)-S(1)	128.0(4)
C(6)-C(1)-S(1)	116.2(5)	C(6)-C(1)-C(2)	115.6(6)
C(3)-C(2)-C(1)	127.0(6)	C(4)-C(3)-C(2)	118.9(6)
C(41)-C(4)-C(3)	119.1(6)	C(5)-C(4)-C(3)	112.9(6)
C(5)-C(4)-C(41)	128.1(6)	C(6)-C(5)-C(4)	129.4(6)
C(5)-C(6)-C(1)	116.2(6)	C(8)-C(7)-N(1)	110.7(5)
C(14)-C(7)-N(1)	99.5(5)	C(14)-C(7)-C(8)	126.0(5)
C(9)-C(8)-C(7)	116.8(6)	C(10)-C(9)-C(8)	114.2(5)
C(9)-C(10)-S(2)	105.8(4)	C(11)-C(10)-S(2)	120.7(4)
C(11)-C(10)-C(9)	113.7(5)	C(10)-C(11)-O(1)	125.2(5)
C(12)-C(11)-O(1)	105.6(5)	C(12)-C(11)-C(10)	129.0(4)
C(11)-C(12)-N(1)	94.8(5)	C(13)-C(12)-N(1)	106.7(5)
C(13)-C(12)-C(11)	117.3(5)	C(14)-C(13)-C(12)	98.3(5)
C(13)-C(14)-C(7)	110.5(5)		

Hydrogen bond lengths (Å) for $C_{16}H_{21}NO_5S_2$

H(2)-C(2)	0.885(52)	H(3)-C(3)	1.099(65)
H(41A)-C(41)	1.080	H(41B)-C(41)	1.080
H(41C)-C(41)	1.080	H(5)-C(5)	0.914(69)
H(6)-C(6)	1.030(56)	H(7)-C(7)	0.836(65)
H(8A)-C(8)	0.948(48)	H(8B)-C(8)	0.946(62)
H(9A)-C(9)	0.913(61)	H(9B)-C(9)	0.928(78)
H(101)-C(10)	0.872(58)	H(12)-C(12)	0.917(48)
H(13A)-C(13)	1.025(53)	H(13B)-C(13)	0.825(94)
H(14A)-C(14)	1.004(66)	H(14B)-C(14)	0.773(60)

Hydrogen bond angles (deg.) for $C_{16}H_{21}NO_5S_2$

H(2)-C(2)-C(1)	114.8(32)	H(2)-C(2)-C(3)	118.1(32)
H(3)-C(3)-C(2)	132.1(34)	H(3)-C(3)-C(4)	109.0(35)
H(41A)-C(41)-C(4)	106.7(4)	H(41B)-C(41)-C(4)	112.1(4)
H(41B)-C(41)-H(41A)	109.5	H(41C)-C(41)-C(4)	109.6(4)
H(41C)-C(41)-H(41A)	109.5	H(41C)-C(41)-H(41B)	109.5
H(5)-C(5)-C(4)	111.4(41)	H(5)-C(5)-C(6)	119.2(40)
H(6)-C(6)-C(1)	114.5(30)	H(6)-C(6)-C(5)	128.8(30)
H(7)-C(7)-N(1)	113.3(45)	H(7)-C(7)-C(8)	108.1(43)
H(7)-C(7)-C(14)	98.9(43)	H(8A)-C(8)-C(7)	122.6(28)
H(8A)-C(8)-C(9)	91.5(29)	H(8B)-C(8)-C(7)	110.0(32)
H(8B)-C(8)-C(9)	106.9(33)	H(8B)-C(8)-H(8A)	107.0(42)
H(9A)-C(9)-C(8)	94.4(34)	H(9A)-C(9)-C(10)	107.2(34)
H(9B)-C(9)-C(8)	109.2(47)	H(9B)-C(9)-C(10)	114.3(48)
H(9B)-C(9)-H(9A)	116.0(60)	H(101)-C(10)-S(2)	88.3(37)
H(101)-C(10)-C(9)	111.7(36)	H(101)-C(10)-C(11)	114.0(36)
H(12)-C(12)-N(1)	122.1(27)	H(12)-C(12)-C(11)	114.2(28)
H(12)-C(12)-C(13)	102.5(27)	H(13A)-C(13)-C(12)	112.8(29)
H(13A)-C(13)-C(14)	123.7(29)	H(13B)-C(13)-C(12)	110.1(62)
H(13B)-C(13)-C(14)	109.2(61)	H(13B)-C(13)-H(13A)	102.7(66)
H(14A)-C(14)-C(7)	125.2(33)	H(14A)-C(14)-C(13)	95.0(33)
H(14B)-C(14)-C(7)	93.3(41)	H(14B)-C(14)-C(13)	129.8(41)
H(14B)-C(14)-H(14A)	106.5(52)		

Selected non-bonded distances (Å) for $C_{16}H_{21}NO_5S_2$

Intramolecular:

C(12)-S(1)	2.330	C(7)-S(1)	2.661
H(6)-S(1)	2.783	H(7)-S(1)	2.713
H(12)-S(1)	2.433	O(1)-S(2)	3.371
C(9)-S(2)	2.669	H(9B)-S(2)	2.655
H(101)-S(2)	1.964	O(12)-O(11)	2.501
N(1)-O(11)	2.152	H(6)-O(11)	2.758
H(7)-O(11)	2.436	N(1)-O(12)	2.423
H(2)-O(12)	2.959	H(12)-O(12)	2.408
O(22)-O(21)	2.703	H(9B)-O(21)	2.702
H(101)-O(21)	2.774	O(1)-O(22)	3.230
H(101)-O(22)	2.546	C(1)-N(1)	2.878
C(8)-N(1)	2.650	C(10)-N(1)	2.730
C(11)-N(1)	2.158	C(13)-N(1)	2.383
C(14)-N(1)	2.364	H(7)-N(1)	2.010
H(8A)-N(1)	2.975	H(101)-N(1)	2.766
H(12)-N(1)	2.040	H(13A)-N(1)	2.946
H(14B)-N(1)	2.830	H(12)-O(1)	2.405
H(13B)-O(1)	2.602	H(12)-C(1)	2.912
H(13A)-C(1)	2.504	H(13A)-C(2)	2.595
H(13A)-C(6)	2.985		

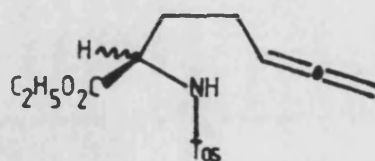
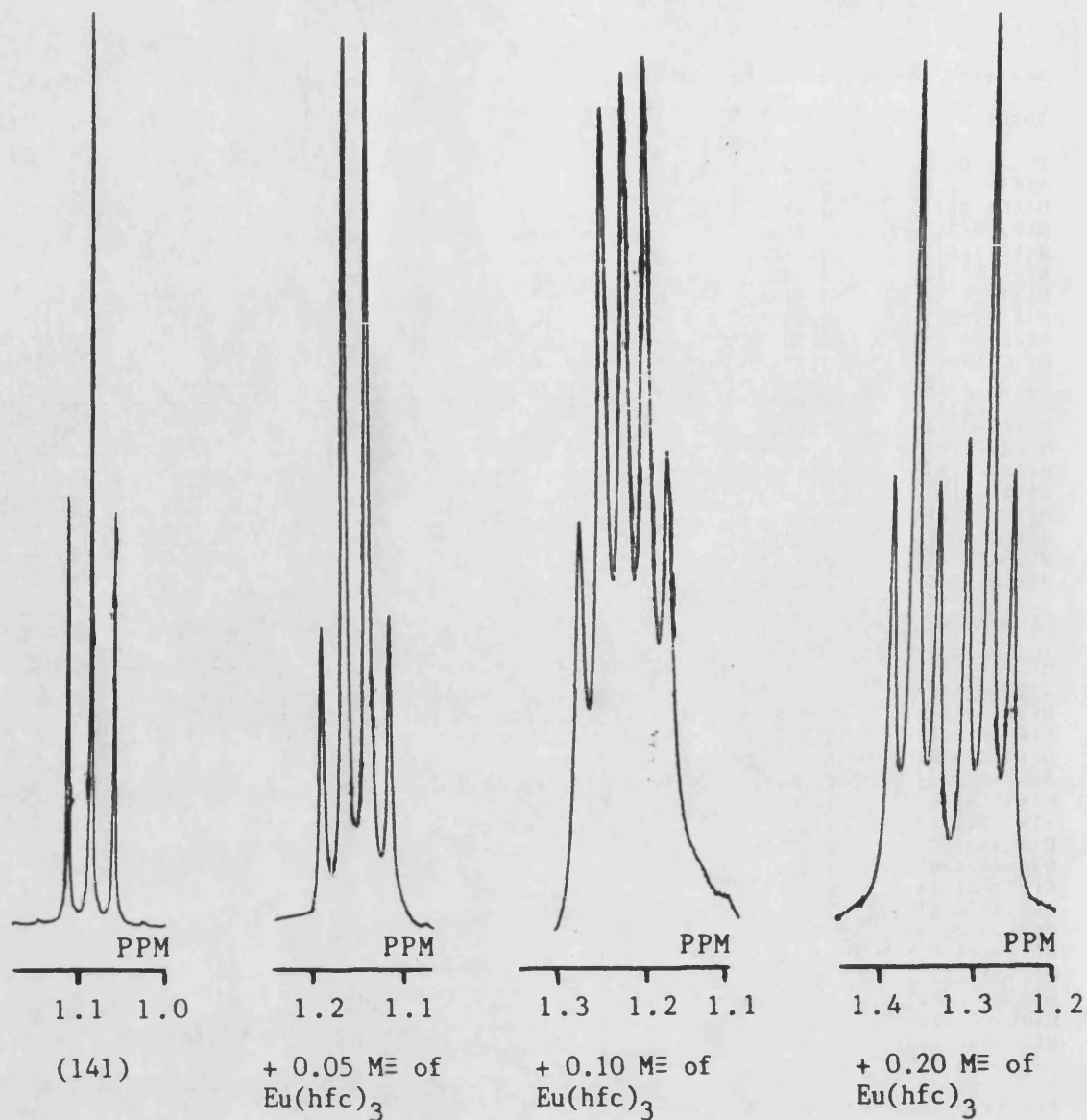
Intermolecular:

H(2)-S(1a)	3.427	O(1)-S(2a)	3.658
C(2)-O(11a)	3.249	H(2)-O(11a)	2.557
H(12)-O(11a)	2.768	C(14)-O(12b)	3.261
H(6)-O(12b)	2.815	H(14A)-O(12b)	2.532
O(1)-O(21a)	3.181	C(15)-O(21a)	3.350
H(3)-O(21c)	2.881	C(41)-O(22d)	3.384
H(41B)-O(22d)	2.862	C(8)-O(22b)	3.403
C(9)-O(22b)	3.219	H(8B)-O(22b)	2.868
H(9A)-O(22b)	2.457	C(10)-O(1e)	3.270
H(8A)-O(1e)	2.692	H(101)-O(1e)	2.558
C(15)-C(3f)	3.104	C(15)-C(4f)	3.353
C(6)-C(41g)	3.381	C(15)-H(41Ah)	3.039
C(5)-H(41Cg)	2.594	C(6)-H(41Cg)	2.489
H(5)-H(41Cg)	2.608	H(6)-H(41Cg)	2.630
H(13B)-C(7i)	2.989	C(13)-C(8i)	3.314
H(13B)-C(8i)	2.628	H(7)-C(13g)	3.034
H(8B)-C(13g)	2.986	H(8A)-C(14g)	2.997
H(13B)-H(7i)	2.502	H(13B)-H(8Bi)	2.217

Key to symmetry operations relating designated atoms to reference atoms at (x,y,z):

- (a) $x, 2.0-y, 0.5+z$
- (b) $x, -1.0+y, z$
- (c) $-1.0+x, 2.0-y, 0.5+z$
- (d) $-1.0+x, -1.0+y, z$
- (e) $x, 2.0-y, -0.5+z$
- (f) $1.0+x, y, z$
- (g) $x, 1.0-y, -0.5+z$
- (h) $1.0+x, 1.0+y, z$
- (i) $x, 1.0-y, 0.5+z$

The distances of atoms bonded to S(2) (i.e. O(21), C(15), O(22)) indicated that there was some mixing of the sites O(21) and C(15) (due to high thermal motion). These were assigned as an oxygen and a carbon atom respectively, as there was more evidence for protons around the atom assigned as C(15). The protons were not shown in (scheme 58) for refinement.



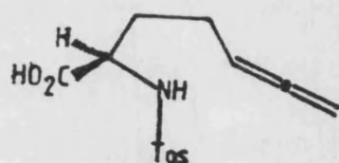
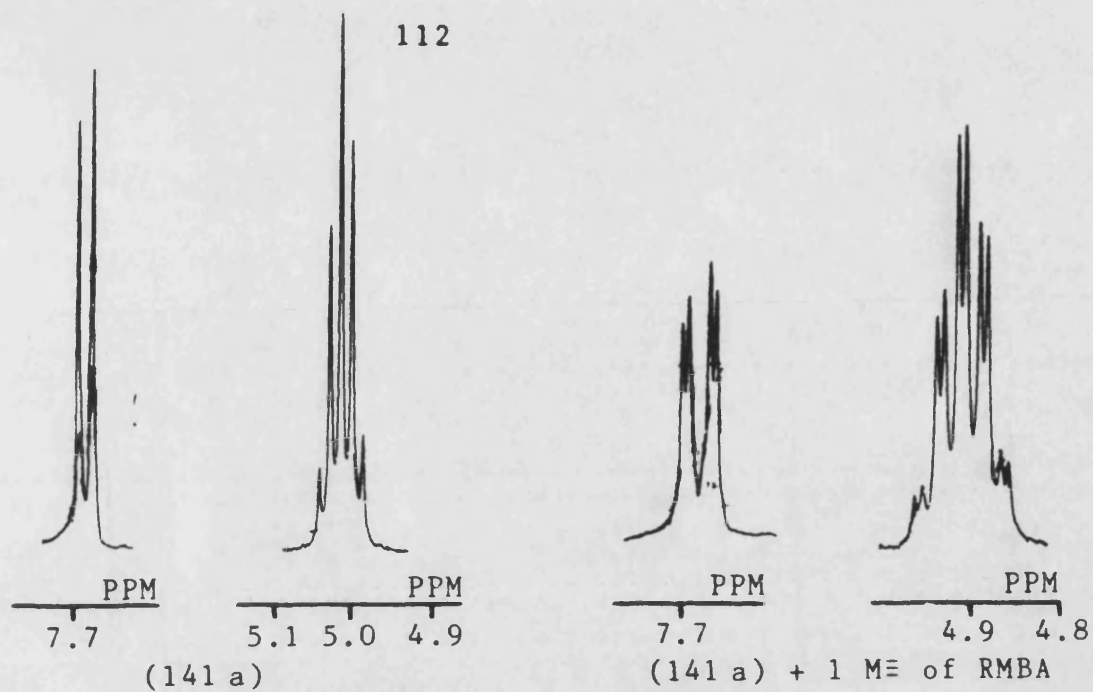
(141) (2R):(2S), 1:1
 (203) (2R)

SCHEME 59

APPENDIX 2

The optical purity of the resolved allenic ester (203) could be assessed by a ^1H nmr chiral shift study. When a ^1H nmr spectrum of a sample of the racemic allenic ester (141) treated with $\text{Eu}(\text{hfc})_3$ was obtained, a splitting of the nmr signals associated with (141) was observed. The splitting was seen due to the resolution of the signals for the different optical isomers of (141). This effect was most noticable for the methyl signal at δ 1.1, which upon sequential addition of $\text{Eu}(\text{hfc})_3$ was gradually resolved from a single triplet into two distinct triplets (scheme 59). Therefore treatment of the resolved allenic ester (203) with 0.2 molar equivalents of $\text{Eu}(\text{hfc})_3$ allowed the optical purity of the sample to be calculated from the relative integral intensities of the two methyl signals at δ 1.28 and 1.36.

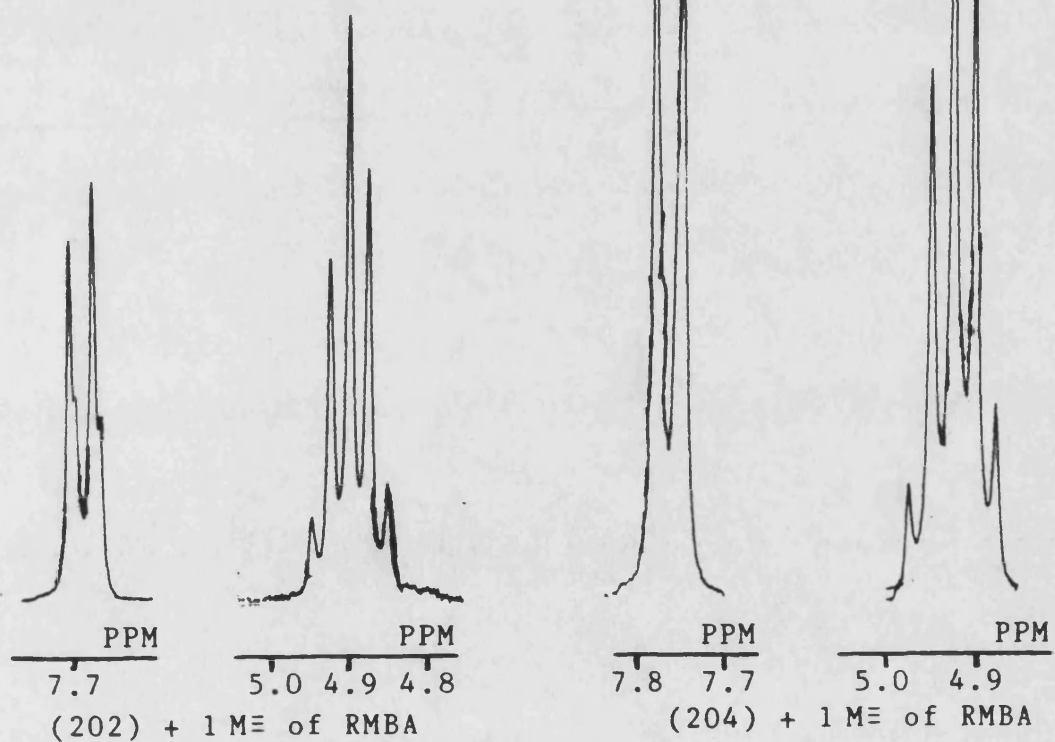
If the ^1H nmr spectra of the racemic acid (141 a) treated with 1 molar equivalent of RMBA was obtained, a splitting of the signals at δ 5.0 and 7.7 was observed. This was due to the amine and the acid forming diastereomeric associated ion pairs, resulting in the partial resolution of some of the signals of the different optical isomers of (141 a). However these signals were not fully resolved and when a mixture of optical isomers was present it was difficult to measure the actual



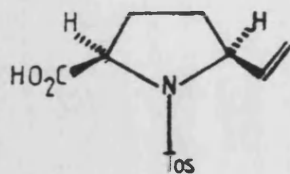
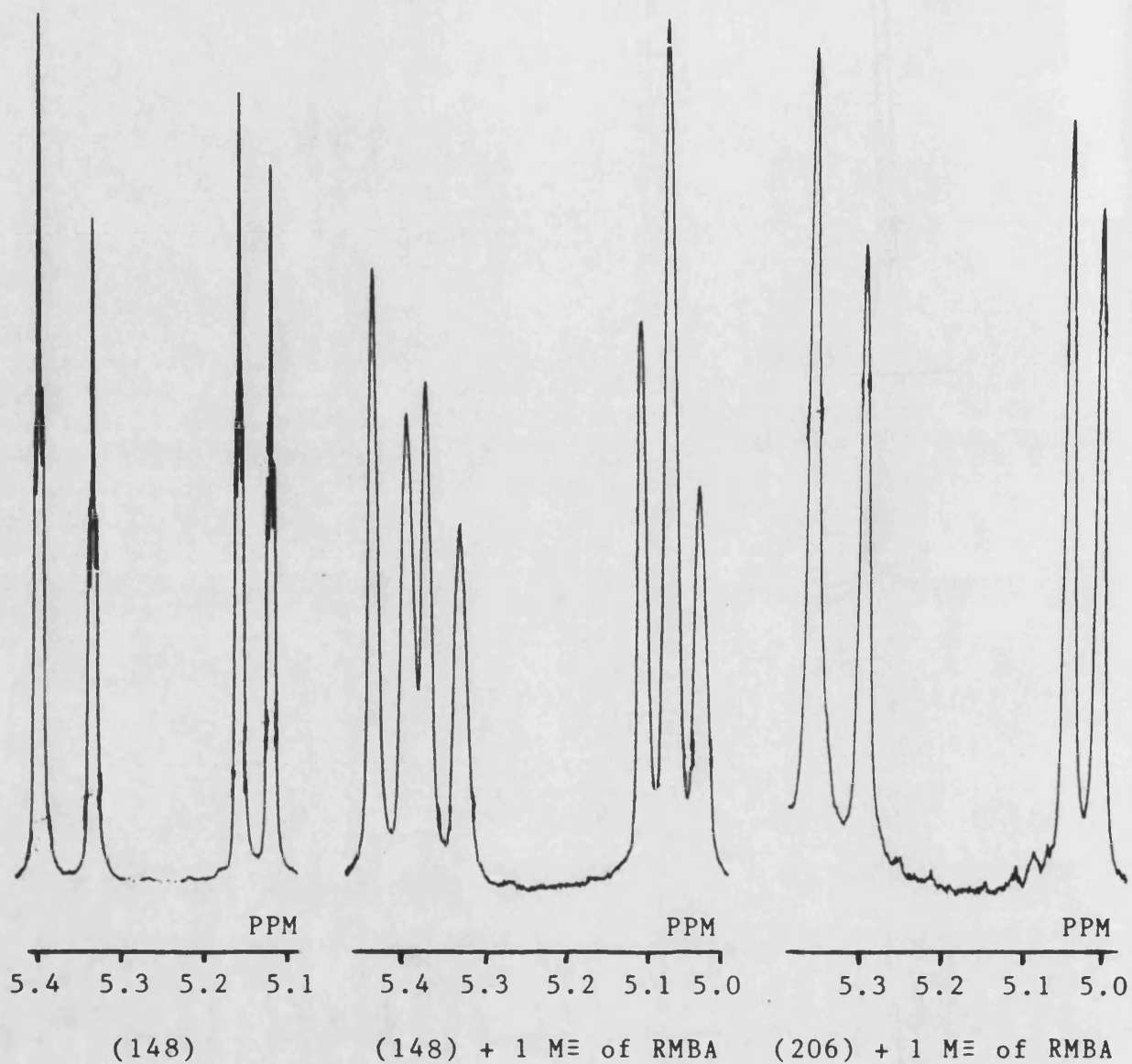
(141 a) (2R):(2S), 1:1

(202) (2S)

(204) (2R)



SCHEME 60



(148) (2R,5S):(2S,5R), 1:1

(206) (2R,5S)

SCHEME 61

isomer ratio. This technique was employed to show that the acids (202) and (204) were optically pure (scheme 60). The ^1H nmr spectra of a mixture of (202), (204) and 1 molar equivalent of RMBA showed a splitting of the signals at δ 4.9 and 7.7. Therefore the acids (202) and (204) were different optical isomers.

This technique was used with greater success to show that the silver induced cyclisation of the optically pure allenic amine (203) gave the pyrrolidine (205) as a single optical isomer. The ester (205) was first hydrolysed to the corresponding acid (206). The ^1H nmr spectrum of a mixture of (206) and 1 molar equivalent of RMBA was then obtained. In contrast to when the racemic acid (148) was employed, in the case of (206) no splitting of the signals at δ 5.0 and 5.4 was observed (scheme 61). Therefore (206) was judged to be a single optical isomer.

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